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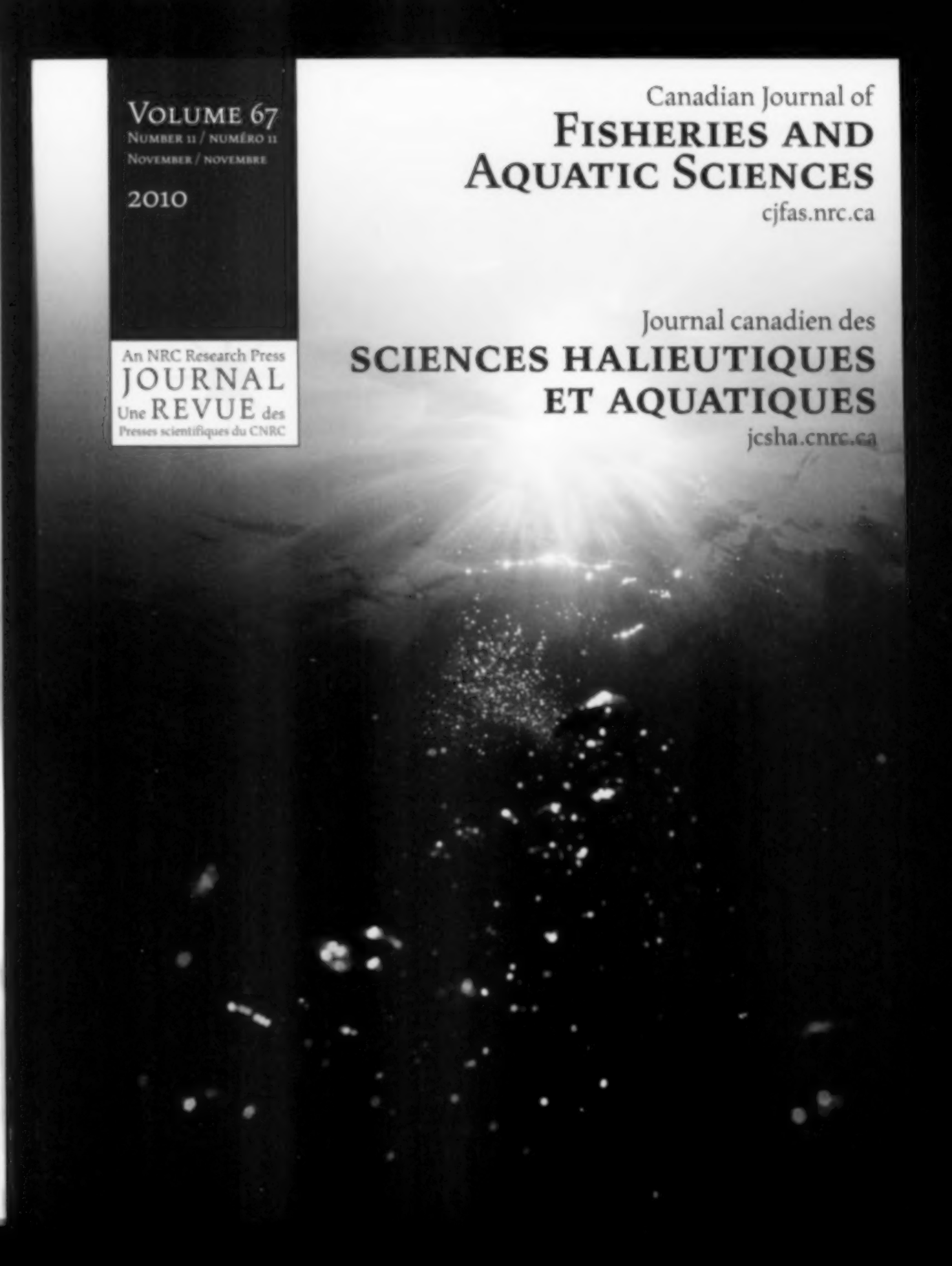

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Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal, and taxonomic differences

Jari Syväranta and Milla Rautio

Abstract: We found considerable seasonal, latitudinal, and taxonomic differences in zooplankton lipid content and concurrent $\delta^{13}C$ values of zooplankton. We collected cladoceran as well as cyclopoid and calanoid copepod zooplankton from boreal and subarctic lakes throughout a year, allowing us to study zooplankton likely subjected to different isotopic fractionation processes and with highly variable lipid contents. Considerable seasonal variation was observed in the difference between bulk and lipid-extracted zooplankton $\delta^{13}C$ values, indicating that seasonally changing lipid content introduced notable variation in zooplankton $\delta^{13}C$ values. The difference between bulk and lipid-extracted material was most amplified in lipid-rich subarctic zooplankton in winter, $\delta^{13}C$ difference being >5 units. Significant differences were also observed among zooplankton taxa, with copepods showing a greater lipid impact on $\delta^{13}C$ than cladocerans. Published lipid correction models failed to produce satisfying fits to our data, and considerable variation was left even after recalibrating the model parameters. This was likely due to taxonomic differences in lipid effects on $\delta^{13}C$ values. We therefore produced separate mass balance-based lipid correction models for cladocerans and also cyclopoid and calanoid copepods. We conclude that arithmetic lipid correction models perform well with zooplankton samples, but taxonomic differences need to be considered.

Résumé : Nous avons observé des différences saisonnières, latitudinales et taxonomiques considérables dans le contenu lipidique et les valeurs associées de $\delta^{13}C$ chez divers taxons zooplanctoniques. Nous avons récolté des cladocères et des copépodes cyclopoïdes et calanoïdes dans des lacs boréaux et subarctiques au cours d'une année complète de façon à étudier le zooplancton sous divers processus de fractionnement isotopique et une forte variation de contenus lipidiques individuels. Des variations saisonnières considérables ont été notées dans les valeurs de $\delta^{13}C$ entre des extraits d'organismes entiers et leurs lipides seuls. Ceci montre que les variations saisonnières du contenu lipidique du zooplancton peuvent influencer les valeurs de $\delta^{13}C$ du zooplancton. D'ailleurs, cette différence de $\delta^{13}C$ entre les organismes entiers et les lipides extraits pouvait atteindre plus de 5 unités pour le zooplancton riche en lipides des milieux subarctiques en hiver. Des différences significatives ont aussi été observées entre taxons zooplanctonique, les lipides des copépodes affectant davantage les valeurs de $\delta^{13}C$ que ceux des cladocères. Les modèles de correction pour les lipides provenant de la littérature ne montrent pas un ajustement satisfaisant avec nos données, même avec une re-calibration de leurs paramètres, ceci étant probablement dû aux différences d'impact des valeurs lipidiques sur le $\delta^{13}C$ entre les groupes taxonomiques. C'est pourquoi, nous avons mis au point des modèles correctifs pour les lipides basés sur le bilan massique séparément pour les cladocères et les copépodes cyclopoïdes et calanoïdes. Nous concluons que les modèles correctifs arithmétiques du contenu en lipides produisent de bons résultats avec des échantillons zooplanctoniques, mais les différences entre les taxons doivent toutefois être considérées.

Introduction

Stable isotope analysis (SIA) is now routinely used in studies of food webs and ecosystem structure. Stable carbon isotopes in particular are commonly used to quantify food sources and energy flow in aquatic ecosystems, since carbon stable isotopes are known to fractionate little between each trophic transfer (DeNiro and Epstein 1978; Peterson and Fry 1987). Stable nitrogen isotopes fractionate more and are typically used to infer trophic positions of consumers in food webs (Minagawa and Wada 1984; Peterson and Fry 1987). While the technical aspects of stable isotope analysis have become easier and more affordable in recent years because of instrumental developments and increased number of commercial SIA laboratories, more concerns are being expressed on how to deal with the inherent variability of stable isotope ratios in sample materials and their many potential sources of error.

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One such potential source of error in SIA is the variable lipid content in sample tissues. Lipids are known to be depleted in the heavier carbon isotope (^{13}C) leading to lower $\delta^{13}C$ values analyzed from tissues with greater lipid content (DeNiro and Epstein 1977; McConnaughey and McRoy 1979; Kling et al. 1992), which can mask the carbon signature of the food source and introduce bias into mixing model calculations or baseline estimates. Especially, temporally variable lipid content can introduce considerable bias into interpretations of $\delta^{13}C$ data because increases in tissue lipid content could result in lower $\delta^{13}C$ values, although the source of carbon (or diet) could remain the same. Lipid content in zooplankton is of particular importance, since some species (such as many copepods) can accumulate considerable lipid storages during the growing season to cope with less productive periods (Kuosa and Gyllenberg 1989; Lee et al. 2006; Kattner et al. 2007), especially in Arctic or subarctic environments, thus potentially reflecting greater lipid-induced variation in their $\delta^{13}C$ values (Grey 2006). Although the lipids in zooplankton are partly incorporated directly from their diet and may therefore be considered as part of their actual diet, another part of lipids is synthesized in situ by zooplankton (Graeve et al. 2005; Kattner et al. 2007) altering the $\delta^{13}C$ values of their body constituents. In addition, since zooplankton accumulate greater lipid reserves than their algal prey, bulk zooplankton $\delta^{13}C$ values may be considerably ^{13}C depleted compared with their diet (Smyntek et al. 2007).

Also, lipids in zooplankton that are accumulated during more productive periods may have a very different $\delta^{13}C$ value than the dietary lipid that the zooplankton is acquiring from diet later in the year. Identifying such temporal variation in zooplankton stable isotope values is of crucial importance to many ecological studies of aquatic ecosystems utilizing SIA, such as those assessing seasonally variable carbon sources to zooplankton (Grey et al. 2001; Karlsson and Säwström 2009). Zooplankton is also an important dietary source to many consumers at higher trophic levels in food webs and is frequently used to establish isotopic baseline levels in the pelagic habitat of aquatic systems (Matthews and Mazumder 2007). Seasonal variation in zooplankton $\delta^{15}N$ values has recently received increasing attention (e.g., Matthews and Mazumder 2007; Syväranta et al. 2008), but less such attention has been given to seasonal variation in $\delta^{13}C$ values (but see Matthews and Mazumder 2005). Although some papers reported no appreciable temporal differences between untreated and lipid-extracted zooplankton $\delta^{13}C$ values (Syväranta et al. 2006; Ventura and Catalan 2008), seasonal increases in lipid storage for overwintering and reproduction purposes could introduce substantial changes on zooplankton $\delta^{13}C$ values (Matthews and Mazumder 2005; Grey 2006), especially in copepods (Smyntek et al. 2007) and in higher latitude regions.

One potential way of accounting for the effects of increasing lipid content on $\delta^{13}C$ is simply to extract the lipids prior to measuring stable isotopes. However, controversial impacts of lipid extraction on nitrogen stable isotope values of sample materials have already been reported (e.g., Sotiropoulos et al. 2004; Ingram et al. 2007; Logan and Lutcavage 2008). If chemical lipid extraction is used for zooplankton, any notable impacts of chemical lipid extraction on sample $\delta^{15}N$ values would then result in doubled analysis costs and extra labor due to preparation of subsamples for lipid extraction.

Greater lipid content in sample materials is typically associated with higher C:N ratios, which can potentially be used to mathematically correct $\delta^{13}C$ values for lipid content (McConnaughey and McRoy 1979), thereby avoiding the need for chemical lipid extraction, such as in chloroform–methanol solution (Bligh and Dyer 1959). Not surprisingly, different models and model revisions to correct the $\delta^{13}C$ values of tissues with variable lipid content have recently received increasing attention in the literature (e.g., Kiljunen et al. 2006; Smyntek et al. 2007; Logan et al. 2008). However, most published papers have concentrated more on the lipid effects on $\delta^{13}C$ values of fish tissues, while invertebrates and zooplankton in particular have received notably less attention. Smyntek et al. (2007) proposed a revised mass balance model (Fry et al. 2003) for lipid correction of zooplankton samples, but this model remains virtually untested. We can expect that models designed for lipid corrections in fish muscle tissues may not perform well on invertebrate samples, which are typically analyzed whole, because of differences in tissue compositions (e.g., chitin in many invertebrates). We might also expect some differences in body compositions between different invertebrate and zooplankton taxa, which could ultimately mean that one model, even if particularly designed for zooplankton lipid correction, may not always perform well if the studied taxa is very different from the one used for model calibration.

Here we present data from seasonal collections of zooplankton samples covering a wide latitudinal gradient from boreal lake systems (central Finland) to subarctic lakes (northern Finland). Our aims were (*i*) to evaluate the impact of seasonally varying lipid content on freshwater zooplankton $\delta^{13}C$ values, (*ii*) evaluate the influence of increasing geographical latitude on zooplankton lipid impacts on $\delta^{13}C$ values, and (*iii*) test the validity of existing mathematical lipid correction models on different zooplankton taxa using a data set that covers one of the largest reported ranges of zooplankton lipid content (20%–62%) and C:N ratios (3.8–19.3) studied for $\delta^{13}C$ values.

Materials and methods

Zooplankton samples were collected in 2005–2007 from four lakes in central boreal Finland near the city of Jyväskylä (62°15′N, 25°46′E, ca. 95 m above sea level) and in 2007–2008 from Lake Saanajärvi in northern Finland near the Kilpisjärvi village (69°05′N, 20°52′E, ca. 670 m above sea level). Samples were collected during open water season (May–November) in lakes in the boreal sampling area by sampling two lakes one to two times a month and two other lakes only in spring and autumn. Mean monthly values for each lake are used in this paper. In the subarctic sampling area, samples were taken four times in winter from under ice (November–May) and four times in open water season (June–October). Zooplankton was sampled by several vertical hauls with zooplankton nets (50–250 μm), and replicate samples were collected during each sampling period from different sites. Samples were then brought to the laboratory

and left overnight in clean tap water or to GF/F filtered lake water to allow gut evacuation. The next day these samples were identified and sorted. Samples from the boreal sampling area were sorted into cladocerans and cyclopoid and calanoid copepods, calanoid copepods being mostly *Eudiaptomus graciloides*. Samples from northern Finland were sorted similarly, but almost all samples of cladocera were *Daphnia umbra*, cyclopoids were all *Cyclops abyssorum*, while calanoids were all *E. graciloides*. After sorting, the samples were stored frozen (–20 °C) in Eppendorf vials and subsequently freeze-dried. These dried zooplankton samples were divided in two to create an untreated sample for SIA and a subsample for lipid-extracted SIA sample. Chemical lipid extraction was done using a 2:1 chloroform–methanol (*v*/*v*) solution (Bligh and Dyer 1959) and 24 h extraction period. The resultant lipid-free material was then dried in an oven at 60 °C and analyzed for comparison with untreated samples. Lipid percentage of dry mass (lipid-%) was determined only for zooplankton samples collected from northern Finland by weighing the dried sample material before and after lipid extraction and calculating the lipid content as a weight difference.

All stable isotope analyses were done at the Institute for Environmental Research, University of Jyväskylä, using a FlashEA 1112 elemental analyzer coupled to a Thermo Finnigan DELTA^{plus} Advantage mass spectrometer (Thermo Electron Corporation, Waltham, Massachusetts, USA). Pike muscle tissue was used as a laboratory working standard, and replicate standards were run repeatedly in every analysis to ensure accurate analysis and to allow any required correction for linearity and drift. Stable isotope ratios are expressed as parts per thousand (‰) delta values ($^{13}C/^{12}C$ or $^{15}N/^{14}N$, i.e., $\delta^{13}C$‰ and $\delta^{15}N$‰), referring to the international standards for carbon (PeeDee Belemnite) and nitrogen (atmospheric nitrogen) (Peterson and Fry 1987). Internal precision for standards was always better than 0.2‰ for both C and N in each run.

Influence of increasing lipid content on zooplankton $\delta^{13}C$ values ($\Delta\delta^{13}C$) was calculated by subtracting values analyzed from lipid-extracted material ($\delta^{13}C_{lr}$) from that of bulk material ($\delta^{13}C_{bulk}$). The $\Delta\delta^{13}C$ values were then compared among separate zooplankton taxa (cladocerans and cyclopoid and calanoid copepods) and between boreal and subarctic sampling areas using analysis of variance (ANOVA) followed by Tukey's multiple comparisons. A Box–Cox transformation was applied when needed to achieve homogeneity of variances (Box and Cox 1964). A multiple linear regression method was used to test for the significance of surface (0–2 m) water temperature, taxa, and sampling area (boreal–subarctic) on $\Delta\delta^{13}C$. Simple *F* tests were applied to test for potential increases in $\delta^{13}C$ or $\delta^{15}N$ variances before and after chemical lipid extraction, and impacts of this procedure on $\delta^{15}N$ values of zooplankton was tested using paired *t* tests between lipid-extracted and untreated samples.

To test published mathematical lipid correction models (Table 1) to our data, we first selected the McConnaughey and McRoy (1979) model, which is widely used for lipid normalization of fish tissues and was recently revised by Kiljunen et al. (2006). The most often used lipid correction models for $\delta^{13}C$ values are either based on lipid normalization model (McConnaughey and McRoy 1979; Kiljunen et al. 2006) or mass balance model (Fry et al. 2003; Sweeting et al. 2006; Smyntek et al. 2007). The difference is that the lipid normalization model by McConnaughey and McRoy (1979) first uses C:N ratio of bulk sample material to calculate a lipid factor (*L*)

$$(1)\qquad L = \frac{93}{1 + [0.246 \times (C{:}N_{bulk}) - 0.775]^{-1}}$$

which is then used to calculate $\delta^{13}C$ of lipid-extracted tissue ($\delta^{13}C_{lr}$)

$$(2)\qquad \delta^{13}C_{lr} = \delta^{13}C_{bulk} + D \times \left(I + \frac{3.9}{1 + 287/L}\right)$$

where *D* is the difference in $\delta^{13}C$ between proteins and lipids and *I* is a constant value. The model revision by Kiljunen et al. (2006) estimated a value of 7.018 for *D* and 0.048 for *I* instead of the original 6 and –0.207, respectively.

The second model is simpler and based on mass balance correction (Fry et al. 2003) and was recently recalibrated more specifically for freshwater zooplankton lipid correction by Smyntek et al. (2007). The mass balance model uses both bulk and lipid-extracted C:N ratios with *D* to calculate $\delta^{13}C_{lr}$

$$(3)\qquad \delta^{13}C_{lr} = \delta^{13}C_{bulk} + D \times \left(\frac{C{:}N_{bulk} - C{:}N_{lr}}{C{:}N_{bulk}}\right)$$

where $C{:}N_{lr}$ and $C{:}N_{bulk}$ are the C:N ratios of the lipid-extracted and untreated sample materials. Appropriate $C{:}N_{lr}$ has to be assessed either from the literature or by chemically extracting lipids from a small subsample and analyzing the lipid free C:N value. Smyntek et al. (2007) estimated a *D* value 6.3 and $C{:}N_{lr}$ value 4.2 appropriate for freshwater zooplankton.

The third model is derived from Logan et al. (2008), who also compared different correction methods with chemical lipid extraction on a variety of sample materials. The model assumes a log-transformed relationship between $\Delta\delta^{13}C$ and C:N ratios:

$$(4)\qquad \delta^{13}C_{lr} = \delta^{13}C_{bulk} + \beta_0 + \beta_1 \ln(C{:}N_{bulk})$$

where values for variables β_0 and β_1 were taken from parameter estimates for all invertebrates given in Logan et al. (2008). All previous three methods (eqs. 1–4) assume a nonlinear relationship between the lipid content (i.e., C:N ratios) and the $\Delta\delta^{13}C$. However, Post et al. (2007) found a linear relationship between the C:N ratios and $\Delta\delta^{13}C$ in aquatic organisms. According to Post et al. (2007), the impact of lipids on $\delta^{13}C$ values can be corrected by

$$(5)\qquad \delta^{13}C_{lr} = \delta^{13}C_{bulk} - 3.32 + 0.99 \times C{:}N_{bulk}$$

We tested these models on our zooplankton data and followed published recommendations (e.g., Logan et al. 2008) of calibrating the existing models to the present data. We chose to recalibrate the parameters used in the mass balance model by calculating the appropriate $C{:}N_{lr}$ value from our lipid-extracted zooplankton samples and then iterating a new value of *D* using Marquardt–Levenberg algorithm, which seeks the values of the parameters that minimize the sum of the squared differences between the values of the ob-

Table 1. Summary of lipid correction models compared in this study with details on taxa and C:N range used to develop and calibrate the models.

Reference	Studied taxa	Studied C:N range	Model*	R^2
Kiljunen et al. 2006	14 fish species from marine, freshwater, and brackish water habitats	2.9–6.3	$\delta^{13}C_{lc} = \delta^{13}C_{bulk} + 7.018 \times \left(\frac{3.90}{1+287L}\right)$	0.65
Logan et al. 2008†	10 species of marine and freshwater invertebrates	3.7–10.7	$\delta^{13}C_{lc} = \delta^{13}C_{bulk} - 2.06 + 1.91 \times \ln(C{:}N_{bulk})$	0.03
Smyntek et al. 2007	Freshwater zooplankton (cladocera, cyclopoid and calanoid copepods)	4.7–17.8	$\delta^{13}C_{lc} = \delta^{13}C_{bulk} + 6.3 \times \left(\frac{C:N_{bulk}-4.2}{C:N_{lc}}\right)$	0.54
Post et al. 2007	16 aquatic animals, including reptiles, fish, and invertebrates	3.0–6.9	$\delta^{13}C_{lc} = \delta^{13}C_{bulk} - 3.32 + 0.99 \times C{:}N_{bulk}$	0.00
This study	Freshwater zooplankton (cladocera, cyclopoid and calanoid copepods)	3.8–19.3	$\delta^{13}C_{lc} = \delta^{13}C_{bulk} + 7.95 \times \left(\frac{C:N_{bulk}-3.8}{C:N_{lc}}\right)$	0.86

Note: R^2 values indicate the model fits to the present data (Fig. 4).
*In the model by Kiljunen et al. 2006, the variable L is defined in eq. 1.
†C:N values and model parameters taken from invertebrate data.

served and predicted values. Each model was evaluated against the recalibrated model using the Akaike information criterion, AIC (Akaike 1974), with a second-order correction for small sample sizes, AIC_c (Burnham and Anderson 1998)

$$(6) \quad AIC_c = 2k - 2\ln(L) + \frac{2k(k+1)}{n-k-1}$$

where k is the number of model parameters, and L is the likelihood value for the estimated model. Models were evaluated by calculating AIC_c values for each model based on the fits to our chemically lipid-extracted zooplankton data, with lower AIC_c values indicating better model performance.

We were also able to estimate the values of D directly from zooplankton samples collected from northern Finland, since these were accurately weighted before and after lipid extraction and lipid-% was calculated. The value of D was estimated from

$$(7) \quad \delta^{13}C_{bulk} = f_{lc} \times \delta^{13}C_{lc} + f_{lipid} \times \delta^{13}C_{lipid}$$

which rearranges to

$$(8) \quad \delta^{13}C_{lipid} = \frac{\delta^{13}C_{bulk} - f_{lc} \times \delta^{13}C_{lc}}{f_{lipid}}$$

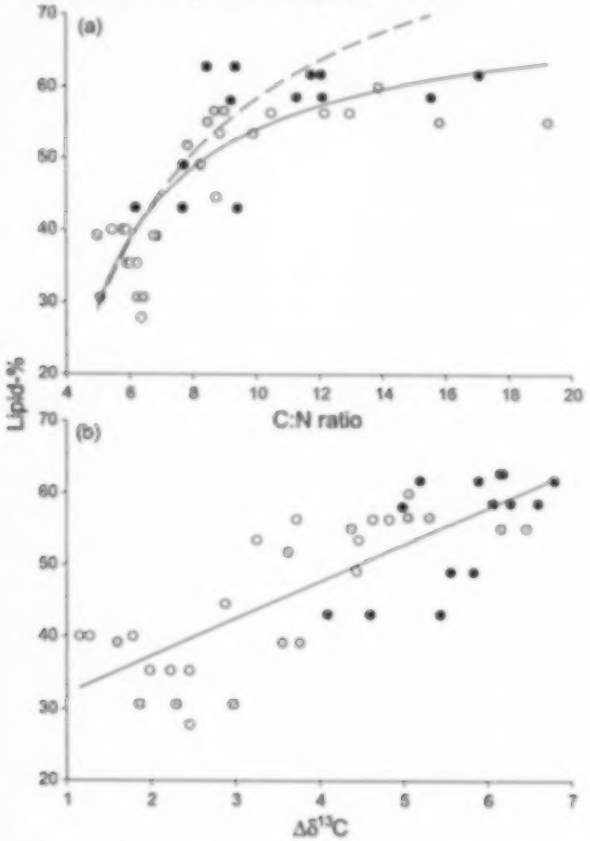
where f_{lipid} is the zooplankton lipid fraction (lipid-%) and f_{lc} is $1 - f_{lipid}$. Values of D for northern Finland zooplankton were then simply extracted from $\delta^{13}C_{lipid}$ and $\delta^{13}C_{lc}$. All statistical analyses and modeling were done using SYSTAT SigmaPlot for Windows v. 10.0 (Systat Software Inc., Point Richmond, California, USA) and SPSS for Windows v. 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Fifty-two zooplankton samples of cladocerans (21) and cyclopoid (20) and calanoid copepods (11) from the boreal sampling area and 51 samples (18, 15, and 18, respectively) from the subarctic sampling area were analyzed for this study. Collecting samples from such different habitats and at different times of year increased the observed range in C:N ratios in our zooplankton samples from 3.8 to as high as 19.3. Lipid-% in zooplankton samples collected from northern Finland varied from ~20% in May to over 60% in early winter, cladocerans typically having the lowest and calanoid copepods (*E. graciloides*) having the highest lipid content. The lipid-% had a clear inverse relationship with C:N ratios of zooplankton and fitting eq. 1 to the observed data resulted in an $R^2 = 0.50$ model fit (Fig. 1*a*). Iterating new parameters to the model using our observed data improved the fit to $R^2 = 0.70$ and resulted in a model to estimate lipid fraction as $72.2/[1 + (0.453 \times C{:}N - 1.562)^{-1}]$. Lipid-% was also linearly related to the difference between lipid-extracted and untreated $\delta^{13}C$ values ($\Delta\delta^{13}C$) of zooplankton ($F_{[1,41]} = 74.9$, $p < 0.001$, Fig. 1*b*).

The $\Delta\delta^{13}C$ values showed significant differences among taxa in both boreal ($F_{[2,39]} = 3.3$, $p = 0.049$) and subarctic sampling areas ($F_{[2,50]} = 11.7$, $p < 0.001$) but were significantly higher in samples collected from subarctic Finland ($F_{[1,91]} = 134.4$, $p < 0.001$). However, only the means of cladocerans and calanoid copepods differed significantly ($p = 0.046$ in southern and $p < 0.001$ in northern areas) in annual $\Delta\delta^{13}C$ values. Zooplankton samples from both sampling areas showed considerable temporal variation in their $\Delta\delta^{13}C$ values (Fig. 2). The $\Delta\delta^{13}C$ value for cladocerans from boreal sampling area ranged from 0.7‰ to 1.4‰ in May–November, while the mean (± standard error, SE) was 1.1‰ ± 0.1‰. Similarly, cyclopoid and calanoid copepods $\Delta\delta^{13}C$ ranged between 0.9‰–1.8‰ and 0.7‰–3.5‰, with mean values of 1.5‰ ± 0.1‰ and 1.9‰ ± 0.3‰, respectively. The variation was more pronounced in samples from subarctic sampling area, where $\Delta\delta^{13}C$ values of cladocerans ranged between 1.8‰ and 4.4‰ (mean 3.4‰ ± 0.3‰) and those of cyclopoid and calanoid copepods between 2.4‰–5.7‰ (mean 4.1‰ ± 0.4‰) and 2.8‰–6.3‰ (mean 5.2‰ ± 0.3‰), respectively. A linear regression indicated that water temperature was an important factor determining the $\Delta\delta^{13}C$ of zooplankton ($F_{[1,39]} = 47.4$, $p < 0.001$, $R^2 = 0.56$; Fig. 3). Adding taxa and sampling site as other explaining factors increased the model performance in explaining zooplankton $\Delta\delta^{13}C$ ($F_{[2,39]} = 37.0$, $p < 0.05$, $R^2 = 0.67$; and $F_{[3,39]} = 47.9$, $p < 0.005$, $R^2 = 0.80$, respectively). All explaining variables (water temperature, taxa, and sampling site) in the final model were highly significant (all $p < 0.005$).

Fig. 1. (*a*) Zooplankton lipid content (lipid-%) in samples from the subarctic sampling area plotted against their C:N ratios. Open symbols represent cladocerans, grey symbols are cyclopoid copepods, and black symbols are calanoid copepods. The dashed line indicates lipid content estimated using the McConnaughey and McRoy (1979) equation to calculate the lipid factor (*L*) shown in eq. 1 ($R^2 = 0.50$). The solid line indicates the eq. 1 fit after iterating new model parameters ($R^2 = 0.70$). (*b*) A linear relationship ($y = 5.2x + 27.02$, $R^2 = 0.65$ is shown between the difference of bulk and lipid-extracted $\delta^{13}C$ values ($\Delta\delta^{13}C$) and lipid content.



Although the mean (± SE) difference between untreated and lipid-extracted $\delta^{15}N$ values was only 0.15 ± 0.07, and therefore biologically negligible, it was statistically significant (paired *t* test; $t_{90} = -2.1$, $p = 0.04$). However, the values were tightly correlated with little scatter along a very considerable range of $\delta^{15}N$ values, and there was no correlation between the C:N ratios and the difference in untreated and lipid-extracted $\delta^{15}N$ values, indicating that any small effect on $\delta^{15}N$ values is likely meaningless. Lipid extraction had no effect on the variance of $\delta^{15}N$ values ($p > 0.05$) but significantly increased the $\delta^{13}C$ variance ($p = 0.024$).

Testing the lipid correction models from Kiljunen et al. (2006), Post et al. (2007), Smyntek et al. (2007), and Logan et al. (2008) to our zooplankton data revealed very different predictions of $\delta^{13}C_{ll}$ from bulk sample C:N ratios. None of the tested models satisfactorily fitted the present data with the original model parameters (Fig. 4, Table 1). Particularly

Fig. 2. Temporal variation in $\Delta\delta^{13}C$ values of different zooplankton taxa in subarctic sampling areas (*a*) and boreal sampling area (*b*). Open symbols represent cladocerans, grey symbols are cyclopoid copepods, and black symbols are calanoid copepods. Mean values (± standard error, SE) are given for replicated samples.

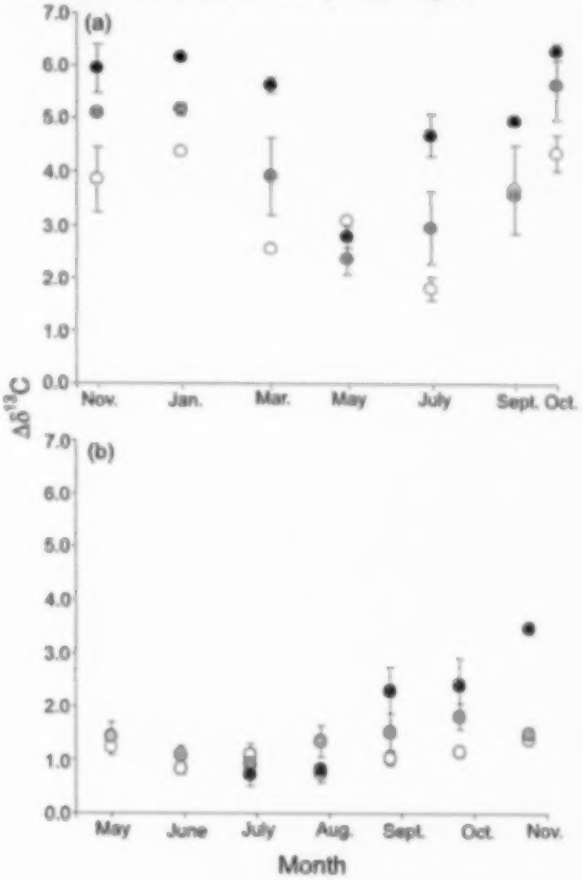
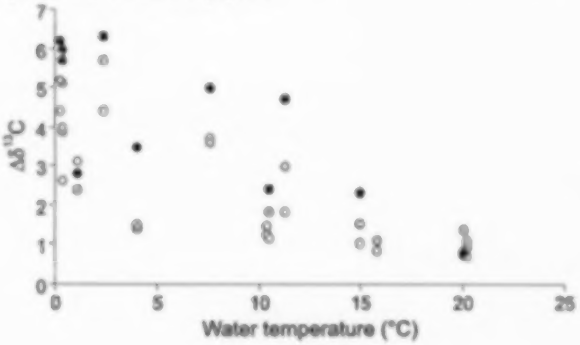


Fig. 3. Relationship between surface (0–2 m) water temperature at the time of sampling and the difference in $\delta^{13}C$ values of bulk and lipid-extracted zooplankton material ($\Delta\delta^{13}C$). Open symbols represent cladocerans, grey symbols are cyclopoid copepods, and black symbols are calanoid copepods.



the linear model by Post et al. (2007) and the log-transformed model by Logan et al. (2008) did not fit to the observed zooplankton data. The models by Kiljunen et al. (2006) and Smyntek et al. (2007) produced predicted $\delta^{13}C_{ln}$ values with generally similar trends but both slightly underestimated the $\Delta\delta^{13}C$. After recalibrating the mass balance model parameters (D and $C:N_{ln}$) according to our data, the model fit improved from $R^2 = 0.54$ by Smyntek et al. (2007) to $R^2 = 0.86$. The calibrated parameters (± SE) of the best fit for the whole data were $D = 7.9 \pm 0.2$ and $C:N_{ln} = 3.8 \pm 0.1$. Some individual samples were excluded when calculating the $C:N_{ln}$ because of incomplete lipid removal ($C:N_{ln} > 5$). As expected, the AIC_c values were lowest, indicating the best fit, for our recalibrated mass balance model, followed by Kiljunen, Smyntek, Logan, and Post models. The two parameters in the Logan et al. (2008) model can also be easily recalibrated using the observed data, seriously improving the model fit ($R^2 = 0.84$ with $\beta_0 = 5.06 \pm 0.4$ and $\beta_1 = 4.30 \pm 0.2$), but the calibrated mass balance model would still have the best performance. Our lipid-% data also allowed a recalibration of the parameters to calculate the lipid factor (L, eq. 1, Fig. 1*a*) for the lipid normalization model. However, this did not notably improve the overall performance of the lipid normalization (by only 9%). But scatter remained even in the mass balance model between the predicted and observed values even after calibrating the model parameters. This scatter was considerably reduced when separate models were applied for individual taxa (Fig. 5). After iterating separate D values for cladocerans ($D = 6.4 \pm 0.2$) and cyclopoid ($D = 7.9 \pm 0.2$) and calanoid ($D = 9.1 \pm 0.2$) copepods, the model fits to individual taxa were $R^2 = 0.84$, $R^2 = 0.94$, and $R^2 = 0.93$, respectively.

Calculating the D values based on $\delta^{13}C_{bulk}$, $\delta^{13}C_{ln}$, and lipid-% of zooplankton collected from subarctic Finland resulted in mean D values (± SE) of 6.4 ± 0.5, 8.2 ± 0.5, and 10.3 ± 0.4 for cladocerans, cyclopoids, and calanoids, respectively. The values for cladocerans and cyclopoid copepods matched closely the D values iterated using the mass balance model for the same subarctic data set (6.4 ± 0.3 and 8.0 ± 0.2, respectively), but was somewhat higher than the iterated value for the calanoid copepods (9.2 ± 0.2) from subarctic Finland. The D values for calanoid copepods ranged from 8.3 to 13.2 but did not reflect any clear pattern, whereas lower D values were calculated from cladocerans and cyclopoid copepods, with lower C:N ratios collected during the summer period.

Discussion

We analyzed three zooplankton taxa (cladocerans and cyclopoid and calanoid copepods) exposed to very different environmental conditions from boreal and subarctic Finland. Sampling from these distinct areas resulted in highly variable body compositions (lipid content) and considerably high C:N ratios (up to 19.3) in our zooplankton samples. Increased lipid content in zooplankton collected from subarctic Finland was clearly reflected as both increased C:N ratios and greater difference between bulk zooplankton and lipid-extracted $\delta^{13}C$ values ($\Delta\delta^{13}C$), allowing a thorough testing of the existing lipid correction models. The relationship between C:N and lipid content was clearly not linear but rather

Fig. 4. Observed relationship between the difference in $\delta^{13}C$ values of bulk and lipid-extracted zooplankton material ($\Delta\delta^{13}C$) and the bulk zooplankton C:N ratio. Solid lines represent the expected relationship based on the lipid correction models tested for the present data; the dashed line indicates the expected relationship based on our recalibrated mass balance correction model.

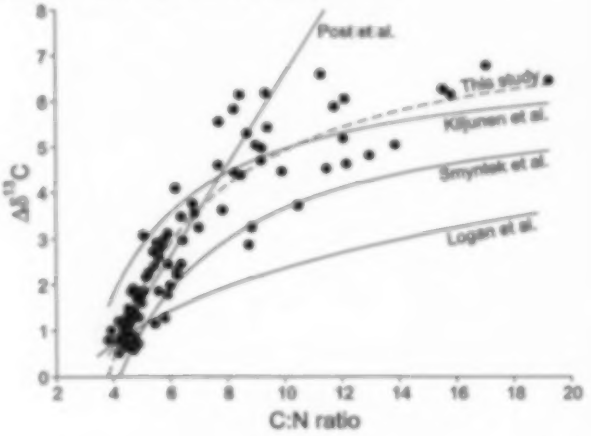
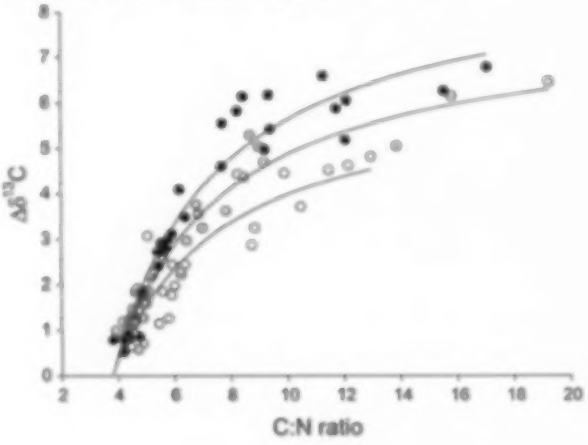


Fig. 5. Mass balance lipid correction models calibrated for separate zooplankton taxa (bottom line, open symbols: cladocerans $R^2 = 0.84$; middle line, grey symbols: cyclopoid copepods $R^2 = 0.94$; top line, black symbols: calanoid copepods $R^2 = 0.93$).



asymptotic, similar to what has been typically reported between C:N ratios and $\Delta\delta^{13}C$ values in literature (e.g., Kiljunen et al. 2006; Smyntek et al. 2007; Logan et al. 2008). Therefore the linear lipid correction model proposed by Post et al. (2007) does not seem appropriate for aquatic samples with higher lipid content and elevated C:N ratios and should not be used for samples with a greater range in C:N ratios than the relatively small range ($C:N < 7$) studied by Post et al. (2007). Within the appropriate range of C:N ratios, the model by Post et al. (2007) does seem to perform equally well to the calibrated mass balance model.

The lipid-% in our samples reached its upper limit (50%–60%) when the C:N ratios were approaching 10. These gravimetrically calculated lipid-% values may be a slight

overestimation because of other constituents such as salts and solids, which are also soluble with chloroform–methanol, and some of them may have been unintentionally removed with the lipid phase. Nevertheless, even if the lipid-% in reality were slightly smaller, the relationship between C:N and lipid-% would still be the same. However, while the lipid-% remained more stagnant after C:N ratios of 10, the C:N ratios continued increasing almost up to 20. Such increase in C:N ratios, but not in lipid-%, might be due to increases in other carbon body parts in zooplankton carapace, which could increase the total carbon concentrations of samples but not total nitrogen or lipid content. But such increases should be evident as larger body sizes of these zooplankton individuals, which did not seem to be the case in our samples. It is perhaps more likely that lipid content around 60% is close to a physiological maximum for many zooplankton species, but the C:N ratios can still be increasing because of assimilation of carbon from diet. The highest C:N ratios in our data were analyzed from samples collected from northern sampling sites in early winter, which would be just after the zooplankton had accumulated energy storages for the winter.

The zooplankton samples analyzed here showed that the $\Delta\delta^{13}C$ varied considerably over time and among taxa. Samples from lower latitude showed notably less variation than those from northern latitude, but the $\Delta\delta^{13}C$ values clearly increased toward autumn and winter in both regions, especially in calanoid copepods with a $\Delta\delta^{13}C$ value up to 3.5‰ in November. This seasonal shift was also reflected as a clear relationship between surface water temperatures and zooplankton $\Delta\delta^{13}C$ values. Water temperature alone explained 56% of the variation in $\Delta\delta^{13}C$ values, but adding zooplankton taxa and sampling area (boreal–subarctic) increased the model performance up to 80%. Water temperature, together with taxa and sampling area, affect the lipid content of the zooplankton, as lipid contents are highest in copepods of the higher latitude region in autumn just before winter and early winter when the water temperatures are low. Samples from the lakes in the lower latitude region were taken only during the ice-free period (typically from May to November), and the increasing $\Delta\delta^{13}C$ values likely reflected increasing lipid storages in zooplankton for the winter period. The ice-free period is much shorter in our subarctic sampling area, typically from late June to late October, but the short open water period was still reflected as notable decrease (over 3‰ in calanoid copepods) in $\Delta\delta^{13}C$ values. The $\Delta\delta^{13}C$ values in zooplankton from the subarctic sampling area were much higher than in our boreal sampling area throughout the year, and in summer the values only briefly dropped to similar levels to those of zooplankton in the south. This greater difference in $\delta^{13}C$ values between bulk and lipid free zooplankton body tissues observed in northern latitudes may not solely be due to greater lipid content of these zooplankters, but the cold climate may also affect the isotope fractionation in lipid synthesis differently because of increased enzymatic fractionation (DeNiro and Epstein 1977; Smyntek et al. 2007). There was also a consistent difference in $\Delta\delta^{13}C$ values among different taxa in both sampling regions, with higher values in copepods than in cladocerans, which can explain the often observed difference in $\delta^{13}C$ values between these taxa (Syväranta et al. 2006; Rautio and Vincent 2007; Smyntek et al. 2007). The highly variable $\Delta\delta^{13}C$ values in both sampled regions indicate that studies using zooplankton $\delta^{13}C$ values to assess baseline levels or especially carbon sources to zooplankton should carefully consider the potential influence of varying lipid content in zooplankton tissues.

Lipid extraction had a significant impact of the $\delta^{15}N$ values of zooplankton in our study. This is contrary to the findings of Ingram et al. (2007). However, although statistically significant, the mean difference of 0.15‰ between lipid-extracted and untreated zooplankton $\delta^{15}N$ values is biologically insignificant, since the typical natural variation among samples is by far greater than 0.15‰ and even the instrument precision may not always reach this accuracy. Since the variation in $\delta^{15}N$ values did not increase after lipid extraction, the lipid-extracted and untreated values were highly correlated along the wide range of $\delta^{15}N$ values, and there was no correlation between C:N value and $\Delta\delta^{15}N$, we can confidently agree with Ingram et al. (2007) that lipid extraction does not significantly alter the zooplankton $\delta^{15}N$ values.

The lipid correction models tested here produced rather unsatisfactory estimates of $\Delta\delta^{13}C$ when using the original model parameters. The AIC_c values were much higher for these models when compared with our calibrated mass balance model. Comparing the individual AIC_c values of each model to the lowest AIC_c value from our mass balance model revealed differences in AIC_c values > 10, basically indicating very little support for these models (Burnham and Anderson 1998). Particularly, the linear model by Post et al. (2007) did not fit to samples with higher lipid content and C:N ratios and therefore should not be used to correct such samples (C:N ratios > 7). Also, the Logan et al. (2008) model with its parameters derived from a variety of invertebrate samples did not fit to the zooplankton data tested here. The invertebrates used to calibrate model parameters were mostly macroinvertebrates in which the higher C:N ratios are more dependent on other body materials (like chitin) than lipids. However, calibrating the two parameters in Logan et al. (2008) model with the present data resulted in improved fit and second-best AIC_c values (the difference to our mass balance model being still 7.7). The models by Kiljunen et al. (2006) and Smyntek et al. (2007) in general provided similar trends and fits to our zooplankton data, but neither model satisfactorily fitted the data when published parameters were used. This was not surprising for the model by Kiljunen et al. (2006), which was calibrated for lipid normalization of fish muscle tissues. However, the lack of fit of the model by Smyntek et al. (2007) specifically calibrated for zooplankton samples was more surprising and is likely due to our samples from subarctic Finland having very high C:N and $\Delta\delta^{13}C$ values.

In addition, our zooplankton samples spanned a very considerable range of C:N ratios (from just 3.8 to 19.3), enabling us to efficiently test different models but to also detect clear among-taxa differences in C:N–$\Delta\delta^{13}C$ relationships, resulting in relatively poor fit for a single model even after recalibration. We chose to use a single $C:N_0$ parameter value (3.8) in all our taxa-specific mass balance models, which was calculated from the whole lipid free zooplankton data and fit perfectly for cyclopoid and calanoid copepod data. However, a slightly higher value might have been

more appropriate for cladocerans, potentially the 4.2 proposed by Smyntek et al. (2007) for all zooplankton. But the data presented here clearly indicate that a single lipid correction model may not perform satisfactorily if the data consist of several taxa with unequal C:N–$\Delta\delta^{13}C$ relationships. Therefore, separate models with taxa-specific parameter *D* values (and potentially also $C:N_0$ values) are clearly needed when analyzing zooplankton samples from such contrasting environments and especially with very high lipid contents and C:N ratios. This conclusion agrees with Logan et al. (2008), who compared chemical and arithmetic lipid correction and presented parameter values for diverse taxa of aquatic fishes and invertebrates. However, when using models with parameters such as *D*, it is worth remembering the underlying assumptions for such parameters. For example, *D* is the difference in $\delta^{13}C$ values between lipids and both proteins and carbohydrates. Contrary to more homogenous tissues like muscle tissue, many aquatic invertebrates including zooplankton are typically analyzed as whole and may contain variable amounts of other materials such as chitin and glycogen (Kiljunen et al. 2006). In other words, the $\delta^{13}C$ of lipid-extracted zooplankton is not the same as $\delta^{13}C$ of zooplankton proteins, as is in the muscle tissue.

The *D* values calculated for samples collected from subarctic Finland were in good agreement with the modeled values for cladocerans and cyclopoid copepods, while slightly higher for calanoid copepods. In general, these values were higher than those presented by Smyntek et al. (2007), who estimated the *D* for different taxa from temperate lakes by directly analyzing the $\delta^{13}C$ of fatty acids. This difference is likely explained by differences in lipid fractionation and synthesis in the colder climate of our higher latitude lake (DeNiro and Epstein 1977). However, our method of calculating the $\delta^{13}C_{lipid}$ from lipid-% is less accurate and may introduce some bias to the final estimates of *D*. Nevertheless, these estimates agreed to our iterated *D* values and showed also some within-taxa variation for cladocerans and cyclopoid copepods. This variation was linked with lower C:N ratios and seasonally to summer period, when the lipid reserves were lowest. Changes in $\delta^{13}C$ of algae and algal lipids that are in part directly incorporated into zooplankton lipids could at this time be quickly reflected as the $\delta^{13}C$ difference between zooplankton lipids and proteins.

The results presented in this paper clearly illustrate how increasing lipid content impacts the $\delta^{13}C$ values analyzed from freshwater zooplankton. However, as concluded in previous studies (e.g., Post et al. 2007), the need for lipid correction only becomes important when the lipid content of samples is variable and (or) high, as indicated by higher (>3.5) and variable C:N ratios. Moreover, all studied zooplankton taxa showed considerable temporal variation in the extent of lipid impact on $\delta^{13}C$ values and in general greater impacts were observed in samples from the subarctic sampling area with colder water temperatures. Equally important is the conclusion that $\delta^{13}C$ of different zooplankton taxa responds differently to increases in lipid content and C:N ratios. This highlights the importance of separating bulk zooplankton samples into different taxa before SIA to minimize observed variation (Matthews and Mazumder 2003; Syväranta et al. 2006). Our results also highlight the importance of calibrating existing models to the present data and using taxa-specific parameters when possible. If appropriate parameters are not available in the literature, we stress the previously published recommendations (Smyntek et al. 2007; Logan et al. 2008) of taking a subsample for chemical lipid extraction to calculate $C:N_0$ and estimate *D*. Preferentially, samples representing the greatest range in $C:N_{bulk}$ should be selected to assure an accurate estimate of parameter *D*. The models can be rather sensitive to different values of *D* (Matthews and Mazumder 2005; Logan et al. 2008; this study), and care has to be taken when deciding the final model parameters.

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Reproductive failure of the red shiner (*Cyprinella lutrensis*) after exposure to an exogenous estrogen

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Abstract: Endocrine disrupting chemicals (EDCs) have been detected in surface waters worldwide and can lead to developmental and reproductive disruption in exposed fishes. In the US Great Plains, EDCs are impacting streams and rivers and may be causing adverse reproductive effects. To examine how estrogenic EDCs might affect reproductive success of plains fishes, we experimentally exposed male red shiners (*Cyprinella lutrensis*) to exogenous 17β-estradiol. We characterized the effects of estradiol on male gonadal histology and secondary sexual characteristics, determined whether exposure reduced reproductive success, and examined the effects of depuration. Adults were exposed to a mean concentration of 70 ng·L^{-1} estradiol, a solvent control, or a water control for at least 83 days. Male exposure to estradiol resulted in elevated plasma vitellogenin concentrations, changes in spermatogenesis, reduced mating coloration and tubercles, altered mating behaviors, and reduced reproductive success with no viable progeny produced. Reproductive endpoints improved upon depuration (28 days). Exposure to estradiol had significant adverse effects on red shiners, indicating that wild populations may face developmental and reproductive difficulties if they are chronically exposed to estradiol.

Résumé : Partout dans le monde, on a pu détecter des produits chimiques perturbateurs endocriniens (EDCs) dans les eaux de surface qui peuvent causer des dérèglements dans le développement et la reproduction des poissons exposés. Dans les Grandes Plaines des É.-U., les EDCs affectent les ruisseaux et les rivières et peuvent produire des effets négatifs sur la reproduction. Afin d'étudier de quelle manière les EDCs œstrogéniques peuvent affecter le succès reproductif des poissons des plaines, nous avons exposé expérimentalement des ides américains à nageoires rouges (*Cyprinella lutrensis*) mâles à de l'estradiol-17β exogène. Nous avons décrit les effets de l'estradiol sur l'histologie des gonades mâles et les caractéristiques sexuelles secondaires, déterminé si l'exposition réduisait le succès reproductif et observé les effets de la dépuration. Des adultes ont été exposés à une concentration moyenne de 70 ng·L^{-1} d'estradiol, à un témoin avec solvant ou à un témoin d'eau pendant au moins 83 jours. L'exposition des mâles à l'estradiol cause une augmentation des concentrations de vitellogénine dans le plasma, des modifications de la spermatogenèse, une réduction de la coloration nuptiale et des tubercules, un changement dans le comportement d'accouplement et un succès reproductif réduit sans production de rejetons viables. Les effets sur la reproduction s'aménuisent durant la dépuration (28 jours). L'exposition à l'estradiol a des effets négatifs significatifs sur les ides américains à nageoires rouges, ce qui indique que les populations sauvages peuvent avoir à faire face à des problèmes de développement et de reproduction s'ils sont exposés à l'estradiol de manière chronique.

[Traduit par la Rédaction]

Introduction

Endocrine disrupting chemicals (EDCs) have been documented worldwide in freshwater, estuarine, and marine environments (Tyler et al. 1998; Mills and Chichester 2005). The physiological effects of EDCs on the reproductive organs and mating behaviors of individual fish are well known and are associated with reduced reproductive capacity (Kime 1995; van der Oost et al. 2003). However, experimental studies connecting EDCs, reproduction, and population declines are rare (Bruno et al. 2003; Jenkins et al. 2009). A notable exception is a whole-lake experiment that showed the addition of an estrogenic EDC at environmentally relevant concentrations led to reproductive failure and a population-level collapse of fathead minnow (*Pimephales promelas*) (Kidd et al. 2007). Laboratory studies linking fecundity with EDC exposure indicate that lower egg production, reduced egg viability, and poor hatching success could correspond to population-level effects (Shioda and Wakabayashi 2000; Zillioux et al. 2001; Nash et al. 2004). Under-

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standing the potential for EDCs to affect fish populations is a critical requirement for conservation and management strategies.

Potential sources of EDCs include wastewater treatment plants (WWTPs), livestock feedlots, and agricultural fields (Jobling et al. 1998; Soto et al. 2003. For example, pharmaceuticals, hormones, and other hormonally active organic contaminants have been documented below WWTPs worldwide (Desbrow et al. 1998; Jobling and Tyler 2003). Many rivers and streams below urban areas are dominated by WWTP effluent (Woodling et al. 2006), and it is a challenge to manage fish populations in these systems. For example, Great Plains rivers in the western United States are influenced by heavy urban and agricultural use (Dodds et al. 2004), contain EDCs, and are home to threatened populations of native fishes. In the South Platte River drainage, EDCs have been detected (Barnes et al. 2002; Kolpin et al. 2002) and fish with intersex gonadal tissues have been observed downstream from WWTP outfalls (Woodling et al. 2006; Vajda et al. 2008). Colorado plains fish populations have been declining for the last two decades (Propst 1982; Nesler et al. 1997), and EDCs may be contributing to these declines.

Further study of the relationship among EDCs, reproductive success, and population processes of native plains fishes is needed to understand if these compounds are contributing to population declines. Recent studies have demonstrated that male fathead minnow and white suckers (*Catostomus commersonii*), species native to Great Plains rivers, become dramatically feminized after exposure to EDCs in the South Platte basin (Vajda 2006; Vajda et al. 2008). Our goal was to test the response of another sexually dimorphic plains fish, the red shiner (*Cyprinella lutrensis*), to an EDC and determine if similar feminization would occur. Additionally, we wanted to investigate the effects of exposure on fecundity. Red shiners are small-bodied, short-lived fish that mature rapidly and naturally spawn in aquaria (Vives 1993). These traits make them good candidates for laboratory studies. Additionally, *Cyprinella* is one of the most abundant and widespread genera of cyprinids in North America, consisting of approximately 30 species (Schönhuth and Mayden 2010). Therefore, inferences regarding exposure to EDCs might be more broadly relevant to many species in the Great Plains and Southwest and provide important insight into how native plains fish species should be managed in relation to endocrine disruption.

We were also interested in the effects of EDCs on courtship behaviors and secondary sexual characteristics in relation to reproductive success. Red shiners are sexually dimorphic, and males display nuptial tubercles and increased coloration during spawning. Alteration in male appearance and behavior may ultimately affect reproduction. Therefore, we chose to focus our research on male responses to exposure and subsequent effects on reproductive success.

Our study was designed to answer three questions involving exposure of the red shiner to the common estrogenic compound 17β-estradiol (estradiol). First, we wanted to know if exposure affected individual male secondary sexual traits and reproductive physiology. Second, we wanted to know if egg deposition, fertilization success, hatching success, and male courtship behavior and components of reproductive capability were affected by exposure to estradiol. Finally, we wanted to determine if removal from chronic exposure to estradiol would allow male red shiners to recover from any diminished reproduction caused by exposure. Fish population responses following depuration were interpreted in light of possible management strategies in the field to reduce the potential for adverse effects of EDCs on the red shiner and other native plains fishes.

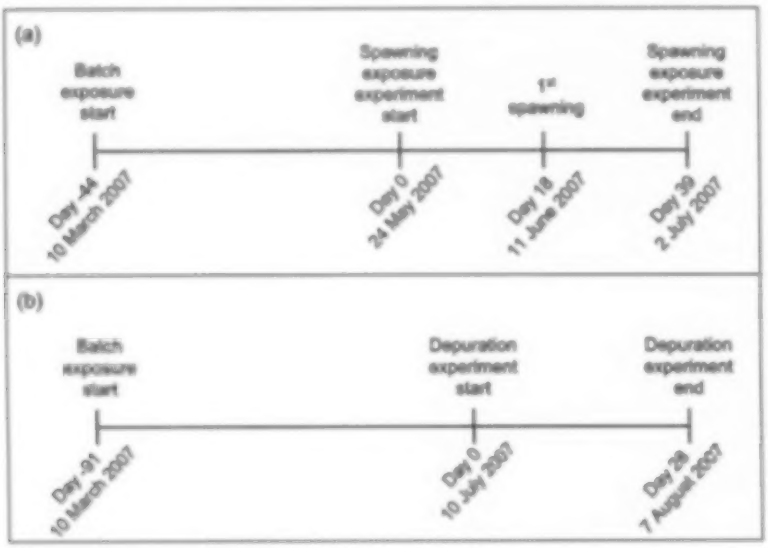
Materials and methods

Our study consisted of two experiments: a spawning exposure experiment and a depuration experiment. The spawning exposure experiment was composed of three treatments: 17β-estradiol (E2, 70 ng·L^{-1} in ethanol), solvent control (SC, 0.00002% ethanol), and water control (WC). We used the naturally occurring hormone estradiol as a surrogate for estrogenic EDCs for the following reasons: it is the major estrogen of female teleosts (Arukwe and Goksøyr 2003), it is necessary for reproduction in male and female teleosts (Nimrod and Benson 1998), it can be found in and downstream of many WWTP effluents, and exposure to exogenous estradiol can induce adverse reproductive effects in diverse fish species (Mills and Chichester 2005). In the United States, aquatic concentrations of estradiol have been estimated to be less than 200 ng·L^{-1}, with a median detectable concentration of 160 ng·L^{-1} (Kolpin et al. 2002). In Boulder Creek, Colorado, concentrations of estradiol have been reported to reach 2.9 ng·L^{-1}, with average and maximum estrogenicity equivalents (accounting for additional estrogenic EDCs) of 29 and 50 ng·L^{-1}, respectively, in the effluent (Vajda et al. 2008). Our goal was to mimic the total estrogenicity seen in Boulder Creek using estradiol. Previous research indicated that actual concentrations could be 10%–30% less than nominal concentrations (Nimrod and Benson 1998). Therefore, we chose a nominal concentration of 120 ng·L^{-1} that would result in a measured concentration similar to the total estrogenicity seen in Boulder Creek.

The experimental setup consisted of a flow-through system of three glass head tanks (20.8 L; one per treatment) that continuously supplied water to glass exposure aquaria (37.9 L) during batch exposure and also to glass spawning aquaria (20.8 L) that would eventually house one male and two females during the spawning exposure experiment (n = 5 per treatment). Male red shiners were batch exposed to E2, SC, or WC for 44 days. Then they were moved to spawning aquaria where they continued exposure and were allowed to spawn with females for 3 weeks (83 days total exposure; Fig. 1*a*). A suite of reproductive endpoints was measured (described below). Batch exposure was logistically necessary to ensure that all male fish within and between each treatment were receiving similar social and environmental cues (light regime, temperature, visual contact with females) to initiate spawning readiness. In addition, red shiners are a social species that occur in large schools, and we felt holding males in isolation for 44 days would cause excessive stress.

The depuration experiment began after 91 days of male exposure to estradiol. This study included a control treatment, where fish from the control aquaria remained in control water (WCWC), and a previously exposed treatment

Fig. 1. Schematic diagram of experimental protocols. Axes are relative and not to scale. In the spawning exposure experiment (*a*), exposure (70 ng·L^{-1} 17β-estradiol, E2; solvent control, SC; water control, WC) began on the date of batch exposure and continued to the spawning experiment end. Reproductive endpoints were measured 21 days after spawning was first seen (first spawning). In the depuration experiment (*b*), exposure (70 ng·L^{-1} 17β-estradiol, E2WC) began on the date of batch exposure and was discontinued immediately prior to the start of the depuration experiment (day 0). Reproductive endpoints were measured 28 days after spawning was first seen, which coincided with day 0.



(E2WC), where fish from the estradiol exposure (70 ng·L^{-1} actual concentration, see above) aquaria were removed and placed into control water. The experimental setup and endpoints measured were identical to the spawning exposure experiment, but instead of continuing exposure, control water was provided to all spawning aquaria beginning on day 0 (Fig. 1*b*). Reproductive effects and spawning were monitored for 4 weeks.

Spawning exposure experiment

Organisms

Adult red shiners were collected by seine from the Arkansas River on the Granada State Wildlife Area, Colorado, USA (38.05°N, 102.23°W), and transported to the Foothills Fisheries Laboratory at Colorado State University (Fort Collins, Colorado) on 29 November 2005 and 9 February 2006. Prior to exposure, fish were held for approximately 8.5 months in 340 L polyethylene round tanks with continuous flows of UV-disinfected well and lake water and a light regime corresponding to the natural photoperiod of the Arkansas River. During the holding period, water temperatures were altered every other day to correspond with Arkansas River seasonal changes, ranging between 6.1 and 23.3 °C. Fish were fed once daily ad libitum with Aquatox commercial flake food (Aquatic Eco-systems, Apopka, Florida) (Viant et al. 2006) throughout holding and experimental portions of the study. Fish used in this experiment were maintained according to guidelines established by the Institutional Animal Care and Use Committee of Colorado State University (approval numbers 06-091A-01 and 06-272A-01).

Test chemical and exposure treatments

A concentrated stock solution for the E2 treatment was prepared at the beginning of the study by dissolving the appropriate amount of estradiol (Sigma, St. Louis, Missouri) in ethanol. The concentrated stock solution was used to make secondary water-based stock solutions every other day by adding 400 μL of solution to 4 L of well water in a stainless steel container. This solution was continuously distributed by a variable speed peristaltic pump at a rate of 0.34 L·min^{-1} through platinum-cured silicone tubing into a glass head tank (20.8 L) and combined with a continuous flow of water (0.14 L·min^{-1}) to produce the E2 treatment concentration. The E2 treatment nominal concentration was 120 ng·L^{-1}; however, the actual waterborne concentration was expected to be 10%–30% of nominal (Nimrod and Benson 1998). A solvent control (SC) treatment was made and distributed in the same manner, adding 400 μL 99% ethanol stock solution to 4 L of well water (0.00002% ethanol). The water control (WC) treatment utilized the same well water. Water from the head tanks of the three treatments was continuously distributed to individual exposure aquaria through Teflon capillary tubes, glass funnels, and Teflon-lined tubing, achieving 99.99% replacement for each aquarium every 24 h. Water chemistry remained constant between treatments over the duration of the experiment (nitrate = nondetectable, nitrite = nondetectable, ammonia < 0.2 mg·L^{-1}, pH = 8, alkalinity = 180 mg·L^{-1}, and total hardness = 300 mg·L^{-1}). Incoming water had a dissolved carbon concentration of 2.84 mg·L^{-1}.

The concentrations of estradiol in each treatment were measured immediately before exposure began and every 1.5 weeks during the exposure period (n = 4) and combined to calculate a mean concentration during the experiment (ac-

tual concentration). A 60 mL syringe was rinsed with methanol (30 mL) and distilled water (30 mL), and then filled with water from each treatment group head tank (60 mL). The water sample was passed through a tC18 cartridge filter (Waters, Milford, Massachusetts) attached to the tip of the syringe. The cartridge was refrigerated until analysis. Water samples were extracted with methanol and analyzed by enzyme-linked immunosorbent assay (ELISA) (Abraxis, Warminster, Pennsylvania) according to the manufacturer's protocols (detection limit 2 ng·L^{-1}, determined by manufacturer).

Exposure conditions of the male red shiner

During the summer holding period, we identified mature males using spawning coloration and held them in a separate holding tank for use in this experiment. After a gradual acclimation to winter conditions (8.5 °C ± 1.2 °C; 8 h light : 16 h dark) and a holding period of 5 months, males were weighed and randomly allocated to three 37.9 L glass aquaria with no spawning substrate. One group of males was exposed to estradiol (E2, described above) and the other two groups received either the solvent (SC) or water control (WC) treatment. Batch exposure lasted 44 days. Female fish were maintained in a separate aquarium with control water. Male exposure continued and temperature was increased by 1 °C every other day until reaching 22.5 °C, where it remained constant for the duration of the experiment (22.4 °C ± 0.42 °C). Photoperiod was increased by 20 min daily until reaching 16 h light : 8 h dark and thereafter remained constant for the duration of the experiment. This temperature and chemical treatment regime was designed to mimic potential exposure during the late spring and early summer spawning seasons in wild populations.

Spawning trials

Fifteen 20.8 L glass spawning aquaria were randomly assigned to one of the three treatments. After 44 days of batch exposure, when a majority of control males displayed spawning coloration and nuptial tubercles, five males were taken from each batch exposure aquarium, weighed, and placed in separate aquaria (n = 5 per treatment, 15 total). Each spawning aquarium contained four spawning stacks placed on four 1.5 cm × 10 cm glass Petri dishes. Individual spawning stacks were made of four 5.1 cm × 5.1 cm sand-colored ceramic tiles. Tiles were drilled through the center, stacked, and held together by a 0.64 cm diameter, 3.8 cm long stainless steel bolt. Tiles were separated by two flat 0.64 cm stainless steel washers and secured by a 0.64 cm stainless steel nut, creating three horizontal crevices averaging 3 mm × 47 mm × 47 mm each (Burkhead and Jelks 2001). After 1 day of male acclimation, females were weighed and added to each spawning aquarium holding one male (two females per male). Exposure continued for 3 weeks after the first sign of spawning. After 3 weeks of spawning, males were immediately removed from aquaria (83 days total exposure), and females were removed the following day. After removal from spawning aquaria, fish were anesthetized with tricaine methanesulfonate (50 mg·L^{-1}). Fish were measured for total length (TL, mm) and weight (g), and condition factor (CF = 100 × [weight (g) × (total length^3 (cm))$^{-1}$]) was calculated.

Reproductive success

Spawning aquaria were monitored at least once daily for egg deposition on the spawning stacks. When eggs were observed, the spawning stack(s) containing eggs were moved to glass beakers containing control water at the experimental temperature. Clean spawning stacks immediately replaced those removed, and all aquaria contained at least one spawning stack at all times. Spawning stacks were held in beakers (21.7 °C ± 1.5 °C) with aeration before processing. Water temperature and presence or absence of fungus on spawning stacks were recorded daily. After 60–72 h, spawning stacks were removed from the beakers and transferred into stainless steel containers (2.7 L) for processing. The numbers of eyed and uneyed eggs were counted and removed from the spawning stack using a razorblade. The total number of eggs counted was used to determine egg deposition, and the number of eyed eggs was used to measure percent fertilization success.

All uneyed eggs and approximately 10% of the eyed eggs were preserved in vials of 10% neutral buffered formalin (20 mL). The remaining eyed eggs were transferred to glass jars with aerated control water (240 mL; 21.7 °C ± 1.6 °C). Observations of hatched or dead fish in each jar were recorded at least once daily. Dead fish were opaque, compared with the translucent appearance of live fish, and were unresponsive to disturbance. Hatched and dead fish were preserved in 10% neutral buffered formalin until all eggs and larvae were removed. Numbers of dead and hatched fish were counted to estimate percent hatching success.

Male behavior

Once visual evidence of spawning was observed, male mating behavior was observed on three occasions, with each observation at least 1 week apart. On each occasion the initial aquarium to be observed was selected randomly and alternated systematically. Male behavior was recorded in all 15 aquaria on each occasion. For each behavioral observation, a digital video camera was placed approximately 0.45 m from the spawning aquarium, enclosed in a black curtain. Each aquarium was recorded for 6 min. When videotapes were reviewed, the final 5 min were used to record behavioral data because other studies suggest waiting at least 1 min after disturbance (Winkelman 1996; Doyle and Lim 2005), and our preliminary observations suggested that red shiners recovered from visual disturbance within 1 min. The frequency of each behavior was measured.

Prespawning behavioral categories included dances, courts, and quivers. "Dances" occurred when the male swam in a circle in front of the stationary female (Ono and Uematsu 1957; Oshima et al. 2003). "Courts" occurred when the male was parallel to the female, facing in the same direction, and tried to push the female into a spawning position (Bjerselius et al. 2001). "Quivers" occurred when the male body undulated or vibrated with fins extended, typically into a crevice (Gale 1986; Moretz and Rogers 2004).

Secondary sexual characteristics

Tubercle number and developmental stage were measured on all male red shiners in the mating experiment, and specimens were preserved in 10% neutral buffered formalin. A microscope (0.8× magnification) connected to Image-Pro

Express (version 5.0.1.26, Media Cybernetics, Bethesda, Maryland) software was used to take a photograph of the dorsal surface of the head, from the posterior edge of the supraoccipital crest to the anterior tip of the snout. Number of visible tubercles was counted, and tubercle developmental stage was scored according to the most highly developed tubercles. The tubercle developmental stages were as follows: (0) no visible sign; (1) visible as white disks; (2) projecting above body surface; (3) prominent but not sharp; and (4) prominent and sharp (Smith 1978).

To document changes in male coloration on the pectoral and caudal fins, digital photographs were taken before preservation of the fish on their left lateral side against a white background in the presence of a color wheel. All images were manually corrected for black and white using Adobe Photoshop 7.0 and analyzed for nuptial coloration with Image-Pro Express software (version 5.0.1.26) to obtain data on mean red, blue, green, and saturation. The trace function was used to select the pectoral fin area (area beginning at the base and encircling the edge of the visible fin), and the caudal fin area (the anterior base of the caudal fin where it meets the caudal peduncle to the posterior edges). Mean color and saturation were determined by selecting the region, producing a color histogram of the image, and recording the reported means that were produced based on abundance and composition of pixels present.

Vitellogenin and histological analyses

After each adult was measured, blood was collected into heparinized capillary tubes via caudal transection (Houston 1990). Samples were centrifuged for 3 min at 11 700 rpm (13 700*g*), and the plasma was stored at –20 °C until assayed for vitellogenin by homologous enzyme-linked immunosorbent assay using an anti-carp kit (Biosense, Bergen, Norway, batch number 0702) in accordance with the manufacturer's protocol. The kit was validated for use with the red shiner by parallelism.

Fish were sacrificed by rapid decapitation and the gonad and liver were removed from the fish and weighed (g) to determine gonadosomatic index (GSI) and hepatosomatic index (HSI), respectively. GSI was calculated as [gonad weight (g) × body weight (g)$^{-1}$ × 100], and HSI was calculated as [liver weight (g) × body weight (g)$^{-1}$ × 100]. All tissues were stored in 10% neutral buffered formalin for histological analysis. Fixed gonads were dehydrated through a graded series of ethanol, cleared in xylene, embedded in paraffin, sectioned (thickness, 6 or 10 μm) using a microtome, mounted on glass slides, rehydrated, and stained with hematoxylin and eosin (Presnell and Schreibman 1997; Blazer 2002). Gonadal sections were examined under a light microscope for stage and maturation of each individual without knowledge of treatment.

Gonadal staging was done using methods utilized by Pawlowski et al. (2004) and Vajda et al. (2008). Sperm abundance within seminiferous tubules of mature males was classified as presence or absence, where 0 = sperm absent and 1 = sperm present. Staging of ovarian sections was based on Blazer (2002) and was as follows: 1 = previtellogenic, 2 = early vitellogenic, 3 = mid-vitellogenic, and 4 = late vitellogenic. Previtellogenic ovaries were identified by a lack of yolk globules and a centrally located nucleus; early vitellogenic oocytes were identified by densely stained yolk globules present throughout; mid-vitellogenic oocytes were identified by presence of yolk globules and center yolk fusion; and late vitellogenic oocytes were identified by yolk fusion extending to the thick, vitelline envelope (Blazer 2002). Ovaries were staged by identifying the most mature oocytes present in the histological section. The sections were then categorized according to relative abundance of previtellogenic oocytes: A (<25%), B (25%–50%), and C (>50%).

Statistical analyses

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc. Cary, North Carolina, USA). Several response variables were log-transformed (coloration, vitellogenin) to meet the assumption of normality. Data were checked for homogeneity of variance across treatments using Levene's test. Parametric data were analyzed using one-way analysis of variance (ANOVA) and are denoted by an F statistic. Both an ANOVA test and a nonparametric alternative, the Kruskal–Wallis test, were used when parametric assumptions were not met. Although we report the χ^2 statistic to be conservative, both ANOVA and Kruskal–Wallis tests always yielded similar main effects probabilities. Therefore, Tukey's honestly significant difference (HSD) method was used to make multiple comparisons for all response variables where the main effects model was significant. For ease of data interpretation in the results, we report statistical significance of the main effects models (F or χ^2 and associated probabilities). In all multiple comparisons, the level of significance used was 0.05. When distributions were tested, Fisher's exact test was used. Behavioral data were assessed for autocorrelation, and when no pattern associated with time was observed, data from all observation events were pooled. Means are reported ± standard error (SEM) and n = 5 unless otherwise noted.

Depuration experiment

Red shiners in the depuration experiment were collected from the Arkansas River site at the same time fish in the spawning experiment were collected and were held in the same manner and exposed to the same water as fish in the spawning exposure experiment. Only fish that were batch exposed to E2WC and to WCWC were used in this experiment as treatments. After 91 days of batch exposure, a total of 10 males (five males from each treatment) were chosen randomly, weighed, and allocated into glass spawning aquaria.

Egg deposition, fertilization success, hatching success, male behavior, secondary sexual characteristics, vitellogenin analysis, and histological parameters were measured in the same manner as the spawning exposure experiment. Water chemistry was measured at the start of the experiment to ensure that estradiol was not detectable in either treatment.

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, North Carolina) in a similar manner to the spawning exposure experiment. Response variables were log-transformed (coloration, percent fertilization success, vitellogenin) to meet the assumption of normality if necessary. Behavioral data analysis utilized only data from the last observation period to represent the ultimate change

over time. Egg deposition, fertilization success, and hatching success data were collected on a daily basis and pooled into four weekly groupings. Egg deposition was analyzed using the general linear mixed model program Proc Mixed for repeated measures ANOVA. Fixed effects were treatment, time, and the treatment × time interaction. Random effects were aquaria within treatment and the residual. Fertilization success and hatching success were analyzed using the generalized linear mixed model program Proc Glimmix with a binomial distribution for repeated measures ANOVA. Fixed effects were treatment, time, and the treatment × time interaction. Random effects were aquarium within treatment and the residual. Post hoc mean comparisons were made using Tukey's HSD method. Means are reported ± standard error (SEM) and $n = 5$, unless otherwise noted. In all tests, the level of significance used was 0.05. To better illustrate the effects of depuration on some response variables, we made comparisons between the depuration and spawning exposure experiments. However, these comparisons should be viewed with caution because the results were from separate experiments.

Results

Spawning exposure experiment

Fish survival was 100% for all treatments during the chemical exposure period. There were no significant differences among treatments in weight, total length, or condition factor of males and of females at the beginning or conclusion of the study (see Supplemental Table S1[1]). Waterborne concentrations of estradiol on all four sample dates ranged from 34 to 160 ng·L^{-1} (mean = 70 ng·L^{-1}, standard deviation (SD) = 60 ng·L^{-1}, $n = 4$, mean 58% of 120 ng·L^{-1} nominal value) and remained relatively constant during the exposure period. Concentrations of estradiol were not detectable in water samples of SC ($n = 4$) and WC ($n = 4$) treatments. There were no significant differences between SC and WC treatment groups in all endpoints, unless otherwise specified.

The mean number of eggs deposited by females in aquaria containing E2 treatment male fish was significantly fewer than aquaria containing SC and WC treatment male fish ($p = 0.0168$, $F = 6.07$; Fig. 2*a*). Percent fertilization success was significantly lower in the E2 treatment than in the WC and SC treatments ($p = 0.0398$, $\chi^2 = 6.45$; Fig. 2*b*). Of the eggs fertilized, the percentage that survived to hatching was significantly lower in the E2 treatment than in the SC and WC treatments ($p = 0.0328$, $\chi^2 = 6.83$; Fig. 2*c*). None of the eggs fertilized in the E2 treatment survived to hatching. Because only three of the five E2 treatment aquaria deposited eggs, $n = 3$ compared with $n = 5$ in the SC and WC treatments with regard to fertilization and hatching success.

Prespawning courtship behaviors were suppressed in males from the E2 treatment (Fig. 3*a*). The frequencies of dances ($p = 0.0078$, $\chi^2 = 9.70$), courts ($p = 0.0183$, $\chi^2 = 7.98$), and quivers ($p = 0.0196$, $\chi^2 = 7.87$) in the E2 treatment were significantly lower compared with those of both controls.

Estradiol had an effect on the secondary sexual characteristics of male fish. The number of tubercles on E2 treatment fish was lower than on SC and WC treatment fish ($p = 0.0083$, $\chi^2 = 9.58$; Fig. 4*a*). Tubercle developmental stage was significantly lower in the E2 treatment than in the SC and WC treatments ($p < 0.001$, Fisher's exact test; Fig. 4*c*). Tubercles seen on E2 treatment fish reached a developmental stage of 1 or below, while SC and WC treatment fish exhibited tubercles at stages 3 or 4. Visually, males showed obvious differences between treatments (Fig. 5). The coloration of E2 treatment males was different than that of SC and WC fish in the pectoral fin and caudal fin regions (Table 1). The pectoral fin of E2 treatment males had higher mean blue ($p = 0.0002$, $F = 19.40$) and lower mean saturation ($p < 0.0001$, $F = 32.41$) than those of SC and WC males, mean red was lower than that in WC males, but not SC males ($p = 0.0532$, $F = 3.78$), and green was higher than the SC treatment, but not the WC treatment ($p = 0.0311$, $F = 4.70$). The caudal fin of E2 treatment males had higher

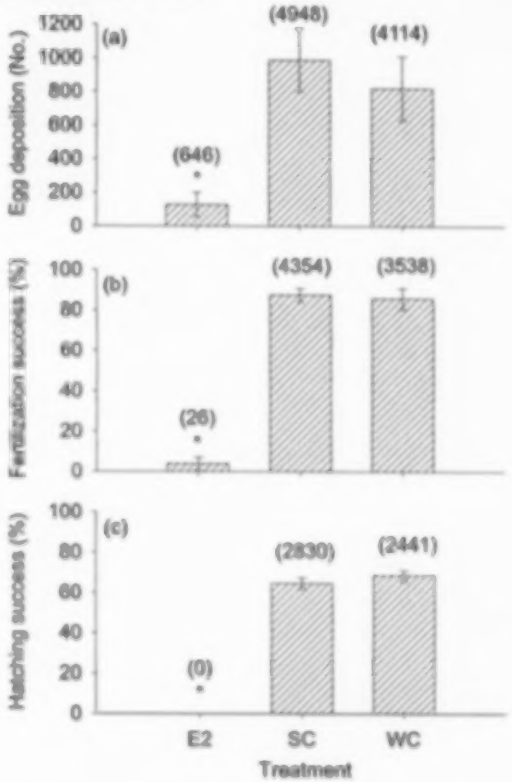
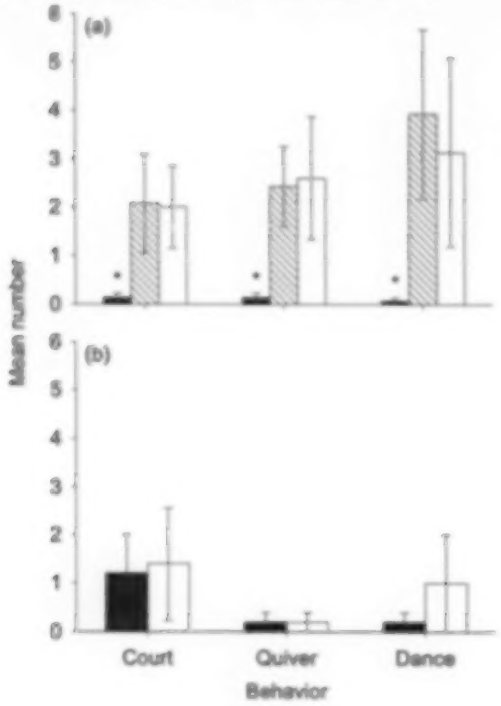


Fig. 2. Mean (± standard error, SEM) egg deposition (*a*), fertilization success (*b*), and hatching success (*c*) of the red shiner (*Cyprinella lutrensis*) after exposure to 17β-estradiol (E2), solvent control (SC), and water control (WC) treatments. Asterisks indicate statistically significant differences from the water control ($p < 0.01$; analysis of variance (ANOVA) and Kruskal–Wallis tests; $n = 5$ for SC and WC treatments and $n = 5$ (egg deposition) and $n = 3$ (fertilization and hatching success) for the E2 treatment). Total eggs per treatment deposited (*a*), fertilized (*b*), and hatched (*c*) are shown in parentheses above each bar.

[1] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 3. Mean (± standard error, SEM) prespawning courtship behaviors of male red shiners (*Cyprinella lutrensis*) in the spawning exposure experiment (*a*), after males were exposed to 17β-estradiol (E2; solid), solvent control (SC; hatched), and water control (WC; open) treatments ($n = 15$ per treatment) and in the depuration experiment (*b*), after males were previously exposed to 17β-estradiol (E2WC; solid) and water control (WCWC; open) treatments ($n = 5$ per treatment). Asterisks indicate statistically significant differences from the water control ($p < 0.05$; Kruskal–Wallis tests).

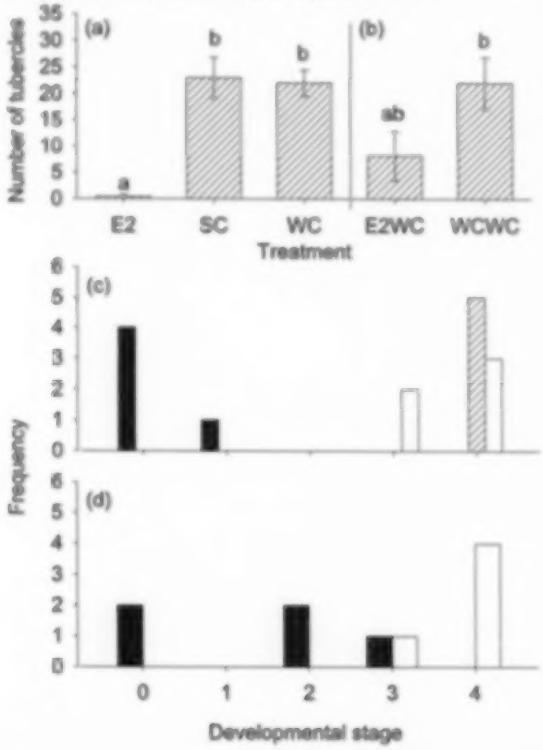


mean blue ($p = 0.0003$, $F = 17.15$) and lower mean saturation ($p = 0.0012$, $F = 12.35$) than those of SC and WC males, higher mean green than the SC treatment, but not WC treatment ($p = 0.0087$, $F = 7.22$), and red did not differ between treatments ($p = 0.5239$, $F = 0.68$); WC and SC did not significantly differ in any test.

Male plasma vitellogenin concentration was significantly higher in the E2 treatment ($p = 0.0090$, $\chi^2 = 9.42$; Fig. 6). Male gonadal stage was significantly reduced in the E2 treatment when compared with SC and WC treatments ($p < 0.0001$, Fisher's exact test). All males in the E2 treatment exhibited stage 0 (sperm absent) gonad development, and all males in the SC and WC treatments exhibited stage 1 (sperm present). Histology also confirmed that exposure to estradiol blocked spermatogenesis and caused regression of seminiferous tubules (Figs. 7*a*, 7*b*).

Plasma vitellogenin concentration was significantly different among female fish that were in aquaria with E2, SC, and WC treatment males ($p = 0.0042$, $\chi^2 = 10.92$); it was lower in females held in E2 aquaria. Female gonadal stage ($p = 0.5615$) and amount of previtellogenic oocytes ($p = 0.1567$) did not significantly differ between treatments (Fisher's exact test). Females in aquaria with males that were exposed

Fig. 4. Male red shiner (*Cyprinella lutrensis*) tubercle number and developmental stage in the spawning exposure and depuration experiments. Fish were exposed for 83 days to 70 ng·L^{-1} 17β-estradiol (E2), solvent control (SC), or water control (WC) treatments in the spawning exposure experiment and for 91 days to 70 ng·L^{-1} 17β-estradiol followed by 4 weeks of depuration (E2WC) and water control (WCWC) treatments in the depuration experiment. Mean (± standard error, SEM) tubercle numbers in the spawning exposure (*a*) and depuration (*b*) experiments are shown. Values with different lowercase letters indicate statistical significance ($p < 0.05$; Kruskal–Wallis test; $n = 5$ per treatment). Frequency of tubercle developmental stage from the spawning exposure (*c*: E2, solid; SC, hatched; WC, open) and depuration (*d*: E2WC, solid; WCWC, open) experiments are shown. Stage was assigned according to the most highly developed tubercles: (0) no visible sign; (1) visible as white disks; (2) project above body surface; (3) prominent but not sharp; and (4) prominent and sharp. Fisher's exact test, $p < 0.0001$, $n = 5$ per treatment.



to estradiol (E2 treatment) exhibited developmental stages 1–3 and females in the control aquaria exhibited stages 2–4, with a majority of females in stage 3 for all treatment aquarium groups. Each treatment had female gonads in categories A, B, and C, with the females exposed to estradiol having slightly more category C, previtellogenic oocytes.

The GSI of E2 treatment males (2.042 ± 0.291) was not significantly different than the GSI of SC males (1.916 ± 0.238) and WC males (1.680 ± 0.144) ($p = 0.5524$, $F = 0.62$). The HSI of E2 treatment males (1.096 ± 0.133) was significantly higher than the HSI of SC males (0.791 ± 0.090) and WC males (0.714 ± 0.054) ($p = 0.0394$, $F = 4.29$). The GSI of females exposed to estradiol in aquaria

Fig. 5. Coloration of adult male red shiner (*Cyprinella lutrensis*) after 83 days exposure to 70 ng·L^{-1} 17β-estradiol (*a*) or water control (*b*) treatments. Solvent control treatment males (not pictured) were visually similar to water control treatment males.

Table 1. Mean (± standard error, SEM) pectoral fin and caudal fin coloration of male red shiners (*Cyprinella lutrensis*) in the spawning exposure experiment and depuration experiment.

Area	Variable	E2	SC	WC	E2WC	WCWC
Pectoral fin	Red	176.10±9.02a	190.36±3.52ab	200.29±4.84b	175.52±5.54	184.22±3.19
	Blue	128.37±19.70a	42.73±4.68b	66.5±5.94b	91.83±18.81*	33.95±2.65
	Green	156.52±13.44a	114.49±6.12b	135.15±7.98ab	134.83±7.28*	101.32±4.05
	Saturation	52.69±14.55a	170.70±8.02b	135.94±8.02b	92.18±26.88*	184.29±5.11
Caudal fin	Red	186.91±6.45a	186.33±3.47a	194.96±6.96a	189.06±1.64	188.37±3.07
	Blue	133.59±7.75a	73.90±7.62b	96.99±6.33b	110.47±16.02	70.12±8.44
	Green	167.88±7.12a	140.51±4.51b	150.92±2.72ab	159.89±7.17*	135.48±3.28
	Saturation	56.05±3.03a	127.01±12.39b	98.71±12.13b	82.39±18.03	127.71±10.89

Note: In the spawning exposure experiment, fish were exposed for 83 days to 70 ng·L^{-1} 17β-estradiol (E2), solvent control (SC), and water control (WC) treatments (values with different letters indicate statistical significance). In the depuration experiment, fish were exposed for 91 days to 70 ng·L^{-1} 17β-estradiol followed by 4 weeks of depuration (E2WC) or water control (WCWC) treatments (asterisks denote significant differences from the control). Analysis of variance (ANOVA) tests, $\alpha = 0.05$, $n = 5$ per treatment.

with E2 treatment males (7.051 ± 1.875) was not significantly lower than the GSI of females in aquaria with SC (11.732 ± 0.711) and WC treatment males (10.096 ± 0.589) ($p = 0.0898$, $\chi^2 = 4.82$). The HSI of females in E2 treatment aquaria (0.741 ± 0.087) was not significantly different than the HSI of females in WC aquaria (0.833 ± 0.056) or SC aquaria (1.270 ± 0.205) ($p = 0.0539$, $\chi^2 = 4.54$).

Depuration experiment

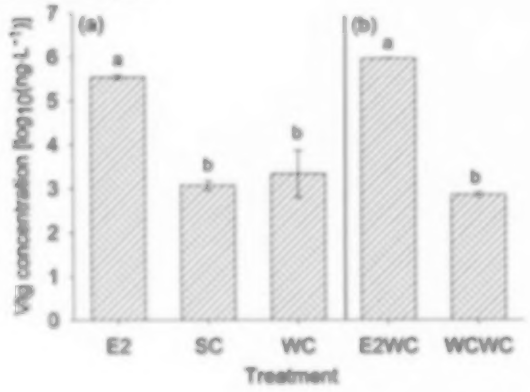
Fish survival was 100% for all treatments. There were no significant differences among treatments in weight, total length, or condition factor of males and of females at the beginning or conclusion of the study (see Supplemental Table S1[3]). Waterborne concentrations of estradiol on all sample dates were below detection limits of the assay. Water chemistry was similar to the spawning exposure experiment and remained constant among treatments over the duration of the experiment.

The mean number of eggs deposited by females differed between the E2WC and WCWC treatments (Fig. 8*a*), especially in the first 2 weeks of the depuration experiment, but overall differences were not statistically significantly different between treatments ($p = 0.0571$) or across the 4 weeks ($p = 0.0585$) (Proc Mixed).

Percent fertilization success was 0% in the E2WC treatment compared with 98% ± 1.1% and 99% ± 0.3% in the WCWC treatment for weeks one and two of the study, respectively (Fig. 8*b*). Fertilization success between treatments was significantly different ($p = 0.0385$, $F = 6.46$); there was not a significant week effect ($p = 0.2825$, $F = 1.45$), and the interaction was significant ($p \leq 0.0006$, $F = 58.78$; Proc Glimmix). Since there was no fertilization or hatching success for the first 2 weeks in the E2WC treatment, the logistic model could not produce exact parameter estimates and the statistics are based on comparisons of the last 2 weeks.

Eggs were not available to hatch until week three in the E2WC treatment, while the WCWC treatment had hatching success of 87% ± 0.1% and 82% ± 0.2% during week one and week two of the study, respectively (Fig. 8*b*). Of the eggs fertilized, the percentage that survived to hatching was not significantly different between treatments ($p = 0.0951$, $F = 4.22$) or weeks ($p = 0.2253$, $F = 24.22$) during weeks three or four (Proc Glimmix; Fig. 8*b*).

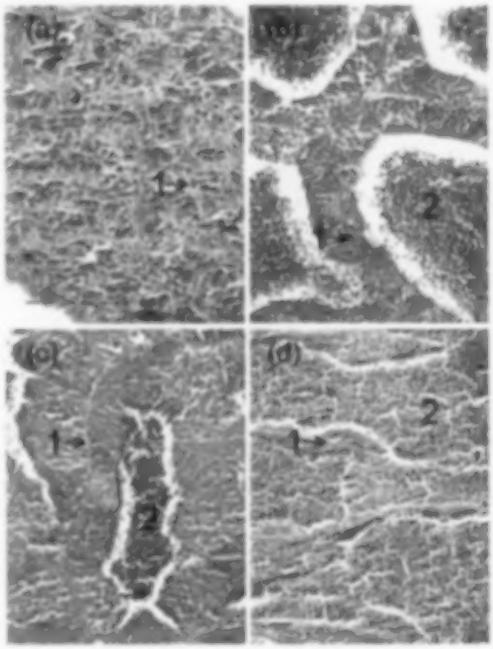
Fig. 6. Mean (± standard error, SEM) vitellogenin (Vtg) plasma concentrations in adult male red shiners (*Cyprinella lutrensis*) at the conclusion of the spawning exposure (*a*) and depuration (*b*) experiments. Fish were exposed to 70 ng·L^{-1} 17β-estradiol (E2), solvent control (SC), or water control (WC) treatments for 83 days (*a*) or exposed to 70 ng·L^{-1} 17β-estradiol followed by a 4-week depuration (E2WC) or water control (WCWC) treatment for 91 days (*b*) before measurements. Values with different lowercase letters indicate statistical significance (analysis of variance (ANOVA) test, $p < 0.05$). The low error associated with the E2WC treatment is not visible in this figure.



Courtship behaviors appeared to recover in males of the E2WC treatment. Frequencies of dancing ($p = 0.8815$, $\chi^2 = 0.02$), courting ($p = 1.0000$, $\chi^2 = 0.00$), and quivering ($p = 1.0000$, $\chi^2 = 0.00$) in the E2WC treatment were not significantly different than in the WCWC treatment (Fig. 3*b*). Secondary sexual characteristics of male fish also showed some recovery. The mean number of tubercles on E2WC fish was not significantly different than those on E2 treatment fish of the spawning exposure experiment ($p = 0.5681$) or the controls of both experiments (SC, $p = 0.0641$; WC, $p = 0.0945$; WCWC, $p = 0.0945$) (ANOVA test; Fig. 4*b*). Tubercles seen on E2WC fish exhibited developmental stages between 0 and 3, while WCWC treatment fish exhibited tubercles at stages 3 or 4 (Fig. 4*d*). The E2WC fish had a significantly lower developmental stage than the WCWC treatment ($p = 0.0476$, Fisher's exact test; Fig. 4*d*). The coloration of E2WC fish was different than WCWC fish (Table 1). The pectoral fin of E2WC treatment males had significantly higher mean blue, higher mean green, and lower mean saturation than fins of WCWC treatment males, with no significant difference in mean red. The caudal fin of E2WC treatment males had significantly higher mean green than those of WCWC males; mean blue was higher and mean saturation was lower in E2WC treatment males, although statistically insignificant. There was no difference in mean red between treatments.

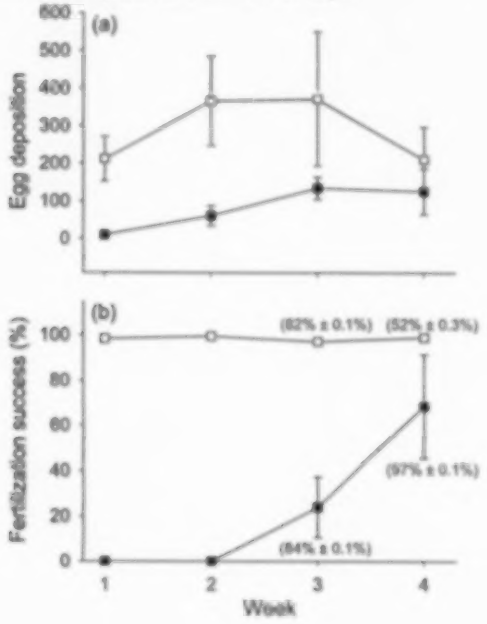
Male plasma vitellogenin concentration in the E2WC treatment was significantly higher than that in the WCWC treatment ($p = 0.0090$, $\chi^2 = 6.82$; Fig. 6*b*). The E2WC treatment was not significantly different than the E2 treatment of the spawning exposure experiment ($p = 0.7292$, $\chi^2 = 0.01$), and the E2 and E2WC treatments were statistically different from the controls in both experiments ($p < 0.0001$, all comparisons; Kruskal–Wallis test). Male gonadal stage was similar between the E2WC and WCWC treatments ($p = 0.4444$, Fisher's exact test), with two E2WC treatment fish in stage 0 and three fish in stage 1 and all WCWC treatment fish in stage 1. Histological examination indicated recovery of spermatogenesis in estradiol-exposed males (Figs. 7*c*, 7*d*). Female gonadal stage ($p = 1.0000$, Fisher's exact test) and plasma vitellogenin concentration ($p = 0.3642$, $\chi^2 = 0.82$) were similar between groups.

Fig. 7. Photomicrographs of representative testicular sections using hematoxylin and eosin staining. Panels (*a*) and (*b*): male red shiners (*Cyprinella lutrensis*) exposed for 83 days to 70 ng·L^{-1} 17β-estradiol (E2, *a*) or solvent control (SC, *b*) treatments. Whereas seminiferous tubules in SC fish were replete with sperm, seminiferous tubules were regressed and spermatogenesis was not evident in E2-exposed males. Panels (*c*) and (*d*): males exposed to 70 ng·L^{-1} 17β-estradiol for 91 days followed by 28 days depuration (E2WC, *c*) or water control (WCWC, *d*). Numbers refer to the outer edge of the seminiferous tubule seminal epithelium (1) and luminal sperm (2). Note the recovery of spermatogenesis in estradiol-exposed fish following depuration. Scale bar: 15 μm.



The GSI of E2WC treatment males (1.398 ± 0.278) was not significantly different than the GSI of WCWC males (1.890 ± 0.155) ($p = 0.1606$, $F = 2.39$). The HSI of E2WC treatment males (0.850 ± 0.231) was not significantly different than the HSI of WCWC males (0.494 ± 0.057) ($p = 0.1770$, $F = 2.19$). The GSI of females in aquaria with E2WC treatment males (9.948 ± 1.381) was not significantly different than the GSI of females with WCWC treatment males (9.771 ± 1.417) ($p = 0.9311$, $F = 0.01$). The HSI of females in aquaria with E2WC treatment males (0.949 ± 0.060) was not significantly different than the HSI of females in aquaria with WCWC treatment males (1.007 ± 0.211) ($p = 0.7989$, $F = 0.07$).

Fig. 8. Mean (± standard error, SEM) egg deposition (*a*), percent fertilization success (*b*), and hatching success (shown in parentheses above and below the treatments, respectively, for weeks three and four) of red shiners (*Cyprinella lutrensis*) in the depuration experiment. Fish were exposed to 70 ng·L^{-1} 17β-estradiol followed by a 4-week depuration (E2WC; solid squares) or water control (WCWC; open squares) treatment for 91 days.



Discussion

Our study clearly demonstrated that estradiol, at an environmentally relevant concentration of estrogenicity, can adversely impact reproductive success of adult red shiners and that reproductive success improved markedly when males were removed from estradiol exposure. Although the effects of EDCs on individual reproductive traits have been well documented, relatively few studies have examined the ultimate reproductive consequences of EDC exposure on fishes (Mills and Chichester 2005), and fewer still documented the recovery of reproduction when exposure ceased (e.g., Nash et al. 2004; Fenske et al. 2005). Our results are a necessary first step in beginning to understand the potential effects of EDCs on population dynamics and persistence, especially in Great Plains ecosystems. Many plains streams are dominated by wastewater discharge and agricultural runoff known to contain EDCs (Woodling et al. 2006). Despite this high potential for exposure, few studies have been conducted to examine individual and population responses of native plains fishes to these compounds. Beyond our study on the red shiner, the only other native plains fish investigated as comprehensively is the fathead minnow (Bringolf et al. 2004; Kidd et al. 2007; Miller et al. 2007). The reproductive decline seen in our study suggests that chronic exposure to EDCs could have severe population repercussions for the red shiner and perhaps other Great Plains fishes but that these impacts could be reversible.

The inability of exposed male red shiners to fertilize eggs could be attributable to impaired spawning behavior, impaired spermatogenesis, decreased milt quality or abundance, or other reproductive disruption resulting from estrogen exposure. Androgens are necessary in spermatogenesis and spawning in fishes (Pandey 1969; Demski and Hornby 1982; Schulz and Miura 2002); exposure to exogenous estrogens can suppress endogenous androgen function through interactions with the hypothalamo–pituitary–gonadal axis (Martinović et al. 2007). Reduced fertility of male fish as a result of exposure to estradiol has been shown in previous studies (Schultz et al. 2003) and is associated with less-developed gonads (Bringolf et al. 2004), delayed spermatogenesis (Billard et al. 1981; Kang et al. 2002), decreased amounts of expressible milt (Bjerselius et al. 2001; Schoenfuss et al. 2002), decreased gonad size (Toft and Baatrup 2001; Bringolf et al. 2004), and reduced sperm motility (Schoenfuss et al. 2002; Casselman et al. 2006). GSI did not differ between treatments in either of our experiments. It appears that exposed males were developing gonadal tissue but, based on histological examination and reproductive success, were not producing functional gametes. Although frequently used as an indicator of EDC exposure, GSI may not be as indicative of an individual's reproductive capability or ultimate reproductive success.

The failure of eggs to hatch when the male parent was exposed to estradiol was likely due to low fertilization success from weakened or dead sperm (Gale 1986). Our results are consistent with previous studies that found decreased hatching success in fish eggs after exposure to an estrogenic endocrine disruptor (Shioda and Wakabayashi 2000; Sohoni et al. 2001; Zillioux et al. 2001). By contrast, other studies did not find an effect of EDCs on hatching success (Thorpe et al. 2003; Fenske et al. 2005), indicating that effects may be species-, compound-, or concentration-specific. The potential for EDCs to interfere with hatching success and thus to reduce recruitment in wild fish populations clearly needs further exploration. Our study suggests that a single estrogenic chemical, at concentrations similar to the total estrogenicity of WWTP effluent, may reduce fecundity of red shiner populations.

Estradiol exposure reduced the number of eggs deposited by females. Reduced egg deposition by females may be due to insufficient stimulatory cues such as decreased mating behavior by the males, reduced secondary sexual traits, or a combination of both. Exposed male red shiners showed fewer courting, dancing, and quivering behaviors than those in control treatments. Other studies that exposed fishes to estrogenic compounds at concentrations comparable to ours have correlated reduced spawning activity and courtship behavior with decreased egg deposition and reproductive success (Bell 2001; Majewski et al. 2002; Martinović et al. 2007). Both coloration and tubercles are thought to be important for male red shiner reproductive success (Matthews 1995). Male red shiners exposed to estradiol had significantly fewer and less developed tubercles and reduced spawning coloration compared with controls. Studies with other fish species also show that males exposed to estradiol had fewer tubercles (Bjerselius et al. 2001; Bringolf et al. 2004) and reduced coloration (Toft and Baatrup 2003; Brion et al. 2004; Kristensen et al. 2005). Despite evidence that es-

trogenic exposure reduces mating behavior, male appearance, and reproductive success, little is known about the repercussions of exposure in wild populations. Our data suggest these changes could have major effects on reproductive output that ultimately may translate to population-level impacts.

Since female fish were exposed to estradiol for a short period of time (39 days) in our spawning exposure experiment, decreased egg deposition could have been due to changes in female reproductive condition. Plasma vitellogenin concentration was significantly reduced in exposed females; however, oocyte stage and vitellogenic oocyte abundance were not, suggesting that exposed females were recruiting oocytes normally. Previous studies have found that female fecundity can be increased with low levels of estrogenic exposure similar to those used in our experiment (Imai et al. 2005; Kristensen et al. 2005). Decreased egg production did not occur until higher concentrations were reached (Imai et al. 2005). Since females in our study were exposed as adults for a short period of time, showed no differences in oocyte numbers from unexposed females, and were exposed at a concentration lower than that found to be detrimental to egg production in other studies (Imai et al. 2005), decreased egg deposition in our experiment was more likely a response to alterations in male spawning cues.

We found that estrogenic effects on most individual male reproductive traits were reversible when male red shiners were removed from estradiol exposure. Mating behaviors and number of tubercles were similar to males that were never exposed. Sperm development did not statistically differ between treatments because three of five previously exposed males developed viable sperm and ultimately reproduced. Exposed males continued to exhibit elevated levels of vitellogenin, and tubercle development remained lower than control males. Egg deposition declined in the control treatments in week four of the depuration experiment, and male reproductive behaviors were lower in this experiment compared with the spawning exposure experiment; however, these results may have been due to declining reproductive activity or capacity. The red shiner is known to have distinct periods of successful reproduction and periods with no evident reproductive success (Durham and Wilde 2005).

Despite the lack of complete recovery in individual reproductive traits and potential declining reproductive activity, the improvement in measures of reproductive success during the depuration experiment was dramatic. Egg deposition in previously exposed treatments increased from 0 in the first week of the experiment to approximately 100 eggs in each tank by weeks three and four. Mean fertilization success increased to 69% after 4 weeks, and mean hatching success increased to 84% in week three and exceeded the control value at 97% in week four. Our depuration study suggests that for red shiners and other plains fish populations that spawn for prolonged periods, an entire reproductive season may not be lost if exposure to EDCs is temporary.

The potential to reverse the effects of chronic exposure suggests potential mitigation or management strategies for fishes during critical reproductive periods. Management strategies could focus on reducing the concentrations of EDCs in aquatic systems through removal of these compounds at WWTP facilities or temporary dilution of effluent by addition of fresh water. Currently, most urban WWTPs are ill-equipped to remove these chemicals and would require changes in treatment processes that presently may not be practical or feasible (Kolpin et al. 2002). Many potential agricultural sources of EDCs are non-point sources that enter streams and rivers from runoff after land application of chemicals and animal waste or seepage and overflow from feedlot operations (Tyler and Routledge 1998; Kolpin et al. 2002). Removal of EDCs in most agricultural settings will require substantial changes in land-use practices to protect surface water and groundwater from agricultural seepage and run-off. Dilution of effluent concentrations could be accomplished by increasing stream flows or reducing effluent discharge. This alternative may improve reproductive success of fish (Robinson et al. 2003), but the efficacy of this strategy depends on the composition of EDCs in the environment (Mills and Chichester 2005) and also on the availability of water, a limited and contentious resource in western Great Plains river basins. Dilution during critical life history stages such as reproduction or development may represent a first step, especially in light of our findings that endocrine disruption in fish exposed as adults may be reversible.

It is important to note that the influence of EDCs on fish reproduction and potential reversibility of exposure depends on EDC concentration and duration, as well as the developmental time period during which the fish are exposed, and may be species-specific (Nash et al. 2004; Fenske et al. 2005; Schafers et al. 2007). To determine how exposure to EDCs could affect particular plains fish populations, knowledge of the normal reproductive biology of each species is critical. The red shiner was intended to serve as a surrogate for other native plains species, especially for native cyprinids or species with similar reproductive strategies, but depending on the interspecific degrees of reproductive complexity, comparisons are not always suitable. Negative effects caused by exposure to estradiol may be intensified in more reproductively complex species, such as those with strong site fidelity for nests or mating territories (e.g., central stoneroller, *Campostoma anomalum*; or centrarchid species). Conversely, effects of EDCs may be reduced in species with less complex mating systems or secondary sexual characteristics. The interaction of life history characteristics with EDC exposure needs to be further explored before predictions can be made about the long-term effects of EDCs on population and community dynamics of Great Plains fishes.

Our study clearly indicates that exposure to estrogenic compounds could dramatically reduce red shiner reproduction, which could have negative implications for recruitment in wild populations. In situ field studies in Colorado suggest that reproductive potential may be compromised because of estrogenic compounds in WWTP effluent. Downstream of WWTPs on the South Platte River and Boulder Creek, white suckers were found with abnormal gonadal development and altered sex ratios with predominantly females in the population (Woodling et al. 2006; Vajda et al. 2008). Adult male fathead minnow experimentally exposed to wastewater from the Boulder Creek facility showed similar changes in secondary sexual characteristics that we observed, including fewer and less prominent nuptial tubercles, less prominent fat

pads, and decreased sperm abundance within the testes than reference fish within 14 days of 50% or 100% effluent. Vitellogenin induction was seen within 14 days in 25%, 50%, and 100% effluent (Vajda 2006). Our findings on how estrogenic chemicals affect the red shiner, a native cyprinid of the Great Plains, complement and validate results of the studies on white suckers (catostomid) and fathead minnow (cyprinid), which are also native.

Many streams and rivers in the Great Plains are dominated by urban and agriculturally derived water (Strange et al. 1999; Woodling et al. 2006) that include compounds known to disrupt reproduction in fishes (Woodling et al. 2006). The problem is not unique to the Great Plains, and it is becoming increasingly evident that many ecosystems worldwide have similar problems associated with human water use and EDCs (Mills and Chichester 2005). Management of these ecosystems will require an understanding of the effects of EDCs on fish populations and communities, as well as mitigation strategies to conserve and protect fishes. Our study is a first step in understanding population-level effects on a common native fish of the Great Plains, shows that red shiner reproductive success is substantially reduced at relevant concentrations of estrogenicity, and suggests that these effects may also occur in wild populations living at or above these concentrations.

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Patterns in apparent survival of Atlantic salmon (*Salmo salar*) parr in relation to variable ice conditions throughout winter

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Abstract: Apparent within-site survival of Atlantic salmon (*Salmo salar*) parr, individually tagged with passive integrated transponders, was not constant throughout the winter period in a 3-year study (2003–2006) in Catamaran Brook, New Brunswick, Canada. Highest decline in apparent survival (19.4%–33.3% of the study population) occurred prior to any ice formation and coincided with early winter acclimatization period (dynamic temperature and discharge regime). Stream discharge and parr maturity were identified to be relevant factors explaining emigration prior to ice formation. Apparent survival was improved during the period affected by subsurface ice and considerably better when surface ice was prevailing, with a decline in population size between 0% and 15.4%. Overall, observed within-site winter mortality was low (4.4%), and the majority of the loss of tagged salmon parr occurred because of emigration. On average, the within-site population of tagged salmon parr declined by 31.7% over the whole winter (November–April). Our data suggest that anthropogenic impacts, like climate change or river regulation, are likely to affect the apparent survival rate and distribution of juvenile Atlantic salmon because of their effects on natural ice regime in streams.

Résumé : La survie apparente dans un même site de tacons du saumon atlantique (*Salmo salar*) porteurs d'un transpondeur passif intégré, ne s'est pas révélée constante au cours de la période hivernale pendant une étude de trois ans (2003–2006) à Catamaran Brook, Nouveau-Brunswick, Canada. Le déclin le plus marqué de la survie apparente (19,4–33,3 % de la population étudiée) se produit avant toute formation de glace et coïncide avec la période d'acclimatation du début de l'hiver (température et régime d'écoulement dynamiques). Nous avons identifié l'écoulement du cours d'eau et la maturité des tacons comme les facteurs pertinents pour expliquer l'émigration avant la formation de la glace. La survie apparente s'améliore durant la période marquée par la formation de glace dans la colonne d'eau et elle est considérablement meilleure quand la glace de surface prédomine; le déclin de la taille de la population varie alors de 0 et 15,4 %. Globalement, la mortalité hivernale observée dans un même site est faible (4,4 %) et la majorité des pertes de tacons de saumons marqués sont dues à l'émigration. En moyenne, la population de tacons de saumons marqués dans un même site a baissé de 31,7 % durant l'ensemble de l'hiver (novembre–avril). Nos données indiquent que les impacts anthropiques, comme le changement climatique ou la régularisation du débit, affectent vraisemblablement le taux de survie apparente et la répartition des jeunes saumons atlantiques par leurs conséquences sur le régime naturel des glaces dans les cours d'eau.

[Traduit par la Rédaction]

Introduction

Winter is recognized to be a critical period for survival of freshwater fishes (Hurst 2007). In fluvial systems, the "winter bottleneck" has typically been associated with energetic imbalance (Finstad et al. 2004*a*; Næsje et al. 2006), lack of suitable habitat (Gibson and Myers 1988), physical disturbance related to ice dynamics (Maciolek and Needham 1952; Cunjak and Therrien 1998), or interaction of multiple stressors (Lemly 1996). Stream salmonids have often been used as model species to study population dynamics during winter, and the estimates of winter loss of juvenile salmonids range from 9% to 100% in natural environments (Huusko et al. 2007). There has been, however, a major problem in conducting winter survival studies because the data collection is very difficult in streams with extensive ice cover. Consequently, most estimates of winter survival are from streams where ice conditions do not preclude the entry to study areas during winter months (e.g., Letcher et al. 2002; Finstad et al. 2004*a*), or the estimates are derived from seminatural settings (Elso and Greenberg 2001; Parrish et al. 2004; Linnansaari et al. 2008). When winter survival is estimated in streams that truly freeze for extended periods, the standard practice has been to resort to a "before–after" approach; that is, the density of fish is estimated in autumn before ice forms and again in spring or early summer when the physical (ice and discharge) conditions re-allow safe and reliable data collection (e.g., Bradford et al. 2001; Lund et al. 2003). Although these studies have been pivotal in providing understanding of population dynamics of juvenile stream salmonids during winter, the relative importance of different periods (e.g., no ice vs. surface ice) of winter for survival has not been adequately assessed in ice-affected riv-

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Table 1. The dates when mean daily water temperature (T) fell permanently below 10 °C (start of before-ice period), freeze-up started (subsurface ice production), and ice breakup occurred during each of the studied winters with the duration of different periods of winter.

Winter	$T < 10$ °C	Freeze-up	Breakup	Before-ice period (days)	Subsurface ice period (days)	Surface ice period (days)
2003–2004	3 Oct.	3 Dec.	10 Apr.	62	9	121
2004–2005	30 Sept.	9 Nov.	2 Apr.	41	17	128
2005–2006	9 Oct.	6 Dec.	3 Apr.	59	11	119

ers. It is well established, however, that the physical environment varies considerably during the course of winter (e.g., Cunjak et al. 1998; Stickler et al. 2009). Also, fluctuations in juvenile stream salmonid physiology (e.g., energy metabolism) occur in different periods of winter in response to altered environmental conditions with possible bearing on survival (Cunjak 1988; Finstad et al. 2004*b*; Næsje et al. 2006). Furthermore, the current climate change scenarios predict that drastic changes in ice regime of rivers are likely to occur (Prowse and Beltaos 2002; Prowse et al. 2006). To predict how juvenile stream salmonid populations may be affected by such changes, the effects of different periods of winter on population dynamics must be explicitly understood.

In this study, we followed individually tagged Atlantic salmon (*Salmo salar*) parr during three winters to identify any variation in apparent survival between different periods of winter in an ice-affected stream. Further, we wanted to establish whether precocious maturity, size of salmon parr (length, mass, or condition factor), stream discharge, and (or) water temperature affect the apparent winter survival within the studied sites.

Materials and methods

Definition of periods of winter in relation to ice conditions

In this article, apparent survival was monitored during three winter periods based on distinctive ice conditions of the stream: (*i*) "Before-ice period" was defined as the time in late autumn when water temperature was <10 °C but no ice had yet formed. The 10 °C threshold was chosen because juvenile Atlantic salmon populations in the studied region exhibit daytime sheltering behaviour when the temperature declines below this value (Rimmer et al. 1983). (*ii*) "Period of subsurface ice formation" was defined as the time when the channel started to freeze and ice processes were dominated by frazil and anchor ice (sensu Stickler and Alfredsen 2009). (*iii*) "Period of surface ice formation" was defined as the time when the surface of the stream was mainly, or totally, covered with solid ice and subsurface ice formation was negligible. Events where ice breakup occurred but a subsequent new surface ice cover formed (i.e., midwinter ice breakup) were also included in the surface ice period.

The winter periods were variable in length during each studied year (Table 1). In addition to the three winter periods, apparent survival could be reliably monitored in three study sites in early spring after ice had melted but before the spring (snowmelt) flood and smolt migration precluded reliable fish tracking, so that an overwinter apparent survival estimate could be calculated.

Study area

The research was conducted over three winters (2003–2006) in Catamaran Brook (46°52′45″N, 66°06′00″W), a third-order tributary to the Little Southwest Miramichi River, situated in central New Brunswick, Canada (Fig. 1). The stream discharge was continually monitored in the Middle Reach of Catamaran Brook, and the discharge is approximately 1.9 times higher in Lower Reach than in the Middle Reach (D. Caissie, Fisheries and Oceans Canada, Gulf Fisheries Centre, P.O. Box 5030, Moncton, New Brunswick, Canada, personal communication, 2009). The mean annual discharge at the mouth of the stream is 1.2 $m^3 \cdot s^{-1}$. The brook represents a typical temperate Atlantic salmon nursery stream that freezes annually for a period of 3–5 months (Table 1). A fish-counting fence situated 250 m upstream from the mouth of the brook is operated from the beginning of May until mid-November and allows the capture of upstream- and downstream-moving fish (Cunjak et al. 1993). Peak smolt migration typically occurs during the second and third week of May. A detailed description of the physical and biological properties of Catamaran Brook can be found in Cunjak et al. (1990, 1993).

The data for this study were collected from four study sites (Table 2). Sites A and C were located in the Lower Reach and sites D and E in the Middle Reach of Catamaran Brook (Fig. 1). All the study sites were relatively shallow (i.e., not deeper than 1 m during normal flow conditions) but consisted of a variety of mesohabitats from riffles to pools (Table 2). Juvenile salmon densities in the actual study sites were not estimated, but the densities in adjacent locations were obtained during the same week when fish tagging occurred (Table 2). It is also important to note that the lengths of the study sites were variable (Table 2), potentially affecting the validity of the proportionate data of fish emigrating out of the study area. However, detailed data on the movements of the passive integrated transponder (PIT)-tagged fish indicated that the salmon parr generally were either extremely sedentary throughout the winter or travelled beyond a scale where nonstandardized study site size could have introduced bias to the encounter rate of fish (Linnansaari 2009).

Tagging and tracking of salmon parr

Atlantic salmon parr were captured using a backpack electrofishing device (LR-24, Smith-Root Inc., Washington, USA). After capture, the fish were anaesthetized with clove oil (40 ppm, ethanol as carrier), and fork length (L_F, nearest mm), mass (accuracy 0.1 g), and maturity (% precocious males) were determined (Table 3). Incision (~4 mm) was then made ~10–15 mm posterior to the pectoral girdle, and a PIT tag was inserted into the body cavity.

Fig. 1. Location of Catamaran Brook in the Miramichi River basin in New Brunswick, Canada (*a*, *b*) and the study site locations (A, C, D, and E) in Catamaran Brook (*c*). Stream discharge was measured at site D, and a fish-counting fence was situated in the site A. Little Southwest Miramichi River is indicated by L.S.W.

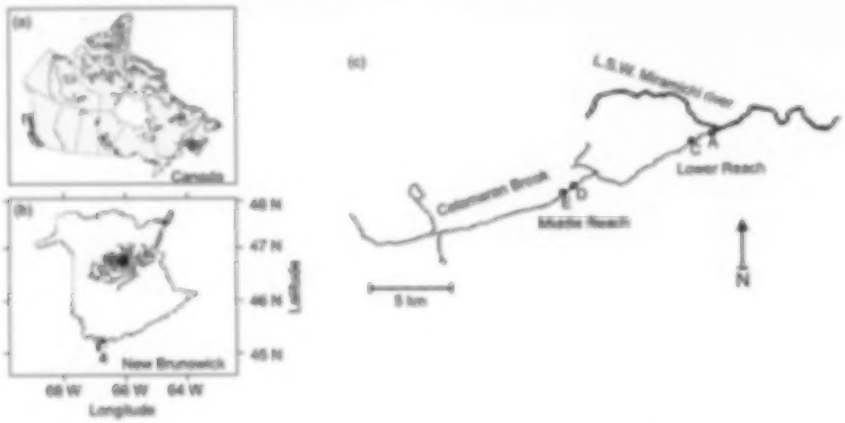

Table 2. Physical properties, juvenile Atlantic salmon density (all age classes), and mesohabitat composition of the four study sites in Catamaran Brook, New Brunswick.

	Study site			
Measure	A	C	D	E
Length (m)	249	103	52	125
Mean width (m)	9.2	10.3	7.5	6.3
Total wetted area (m^2)	2293	1011	383	743
Density (no.·100 m^{-2}± standard error, SE)	69.4±4.8	54.5±0.8	12.7±0.4	27.1±1.4
Distance from river mouth (km)	0.1	1.0	8.5	8.8
Mesohabitat composition (%)				
Run	6	0	0	0
Glide	12	20	30	21
Pool	7	14	9	7
Flat	15	5	13	12
Riffle	60	61	48	60
Total	100	100	100	100

Note: Mesohabitat classification is based on Borsányi et al. (2004) but modified to be better suited to small streams. All habitats with broken surface pattern (wave height > 4 cm) were termed as "riffle"; the habitats with smooth surface pattern but steep gradient (>4%) were termed as "run"; and the habitats with smooth surface pattern but moderate gradient were termed as "glide" (surface velocity > 0.5 $m \cdot s^{-1}$), "flat" (surface velocity < 0.5 $m \cdot s^{-1}$, depth < 0.4 m), or "pool" (surface velocity < 0.5 $m \cdot s^{-1}$, depth > 0.4 m).

Parr were tagged using 23 mm (0.6 g in air) or 32 mm (0.8 g in air) PIT tags (RI-TRP-WRHP, RI-TRP-WR2B, respectively; Texas Instruments, Texas, USA). The 32 mm tags were used only during the winter of 2005–2006 and only for fish larger than 100 mm L_F (n = 19). The incision was closed with a single suture (Monosof black nylon, 6–0 taper, C-1 cutting needle). After tagging, salmon parr were held in large, substrate-filled containers within the study sites and released back to the stream the following morning. The number of fish tagged varied in each study site (Table 3), and the tagging took place from late October to early November (Fig. 2).

Tagged fish were monitored with passive (stationary) and active (portable) antennae using a Texas Instruments Radio Frequency Identification Series 2000 system. The stationary antenna data were used to relate the exact timing of emigration to physical (stream) parameters, whereas the data from active tracking surveys were used to confirm the number of fish remaining in the study sites and their status (dead or alive).

The stationary antennae were set-up as swim-through gates (7.3–8.6 m in width and 0.5–0.6 m in height), and two antennae were used in sequence to detect the direction of fish movements. The reader system was powered with two 12 V DC deep-cycle marine batteries (in parallel) that were replaced every 3 days. The antennae were tested using a "dummy" tag each time before the batteries were changed to ensure that the antennae had been operational and no data had been lost. The stationary antennae were in use from November to December 2003 at the downstream end of site A and from November 2004 to March 2005 at the downstream and upstream end of site C.

Active tracking with the portable system was carried out mainly using a one-person antenna. Details on construction, accuracy, and efficacy of the antenna have been described previously (Roussel et al. 2000; Linnansaari et al. 2007).

Table 3. Number of Atlantic salmon parr tagged (n) at each study site, with description of mean fork length (L_F), mass, tag-to-fish mass ratio (W_T/W_F), and maturity of the study populations.

	Study site (year)					
	A (2003–2004)	C (2004–2005)	C (2005–2006)	D (2003–2004)	E (2004–2005)	E (2005–2006)
n	57	36	31	28	23	53
L_F (mm)	93±15	97±15	91±16	99±13	115±11	89±11
	(78–140)	(75–137)	(79–127)	(83–130)	(87–135)	(73–124)
Mass (g)	9.6±5.7	10.4±5.5	8.8±5.4	12.4±4.1	16.9±4.7	8.8±4.0
	(5.5–31.2)	(5.1–30.1)	(5.1–20.5)	(6.2–24.9)	(6.5–26.2)	(5.1–21.6)
W_T/W_F (%)	7.5±2.5	5.8±2.3	7.0±2.7	8.2±2.0	4.0±1.8	8.2±2.0
	(1.9–10.9)	(2.0–11.8)	(3.9–11.7)	(2.4–9.7)	(2.3–9.2)	(3.7–11.8)
Maturity (%)	18	22	29	11	9	43

Note: Values are reported as mean ± standard deviation (SD), with range in parentheses. In addition to tagged fish shown in the table, five PIT-tagged salmon parr from a former study (Kilburn 2004) were located during the first active tracking survey in the site A during winter 2003–2004 and were subsequently used in the study.

The detection distance was measured to be approximately 65 cm for 23 mm tags and 73 cm for 32 mm tags, when the tag's cylindrical axis was held parallel to the plane of the antenna inductor coil loop (maximum read range for vertically oriented tags 89 cm and 96 cm, respectively). Active tracking was carried out multiple times during each period of winter such that the tagged salmon parr were followed under very diverse physical conditions, ranging from ice-free situation to very severe anchor ice events (5.0%–83.9% of the wetted area covered) and eventually to partial (36.8%–92.5% of wetted area) and full surface ice (100%) cover conditions (Table 4, Fig. 2). A two-person antenna (Linnansaari and Cunjak 2007) was used in April 2006 to survey a kilometre of stream both upstream and downstream from the study site E to determine the extent of movements and the fate of salmon parr that had emigrated during winter.

It was assumed that if a PIT-tagged salmon was present in a study site during a tracking survey, it would be detected. This assumption was based on several factors: (*i*) the chosen study sites were relatively open areas and easy to scan for tags with the portable antenna; (*ii*) the tracking surveys were carried out during a period of relatively cold water temperature when salmon parr have been shown to tolerate tracking operations without fright bias (Linnansaari et al. 2007; Linnansaari and Cunjak 2007); (*iii*) the efficacy to recover tags has been shown to be very high for the antenna types used in this study (Linnansaari et al. 2007; Linnansaari and Cunjak 2007); (*iv*) the tracking was carried out repeatedly (typically 3–13 times) over a 24-h period on each studied date (detailed data on individual trackings are considered elsewhere; Linnansaari 2009); and (*v*) the structure of the resulting data set indicating that fish that were expected to remain in the study site were either found each time (data analysed based on tracking dates, not individual trackings within each date) or were no longer present (i.e., not found within study site in any later tracking). Thus, the encounter rate was generally considered to be 100%. However, exception to this happened on three dates during surface ice period in site E (2005–2006) when the tracking was carried out only during daylight. It was known from the tracking data during before-ice conditions that the daytime shelter of two PIT-tagged salmon parr was situated under a logjam where the daytime detection was not possible if the surface ice was present (owing to insufficient detection distance). These two fish were never detected in daylight if surface ice precluded maneuvering of the antenna, but were always detected adjacent to the logjam if the trackings were also carried out at night, when the fish moved away from the shelter. For these situations (two fish on three dates; $n = 6$), the protocol suggested by Steingrimsson and Grant (2003) was followed. That is, as the two parr were not observed during a particular tracking survey date (daytime data only) but were recovered during both the preceding and the subsequent tracking survey date, the fish were assumed to have remained in the study site. Tag loss was not expected to contribute to fish loss because use of sutures has been shown to effectively eliminate any tag loss (Roussel et al. 2000).

Definitions of ecological measures and statistical analysis

The decline in the number of PIT-tagged salmon parr remaining within the studied sites was reported as apparent survival (sensu Olsen and Vøllestad 2001). The measure represents an underestimate of the true overall survival because the ultimate fate of salmon parr that emigrated from the studied site was unknown (i.e., dead or alive) in most cases. Since the different periods of winter used in this study were variable in duration (Table 1), instantaneous daily loss rates were calculated to facilitate comparisons and to make data more applicable in areas where similar physical conditions occur but the duration of the periods may further vary. Instantaneous daily loss rate (M_i) was calculated for each period as $[\ln(N_{initial}) - \ln(N_{final})] \times (D_{final} - D_{initial})^{-1}$, where N is the number of parr observed alive, and D is day of year. Again, instantaneous daily loss rate cannot be interpreted as a rate of fish mortality but rather as a daily rate to which tagged fish were no longer found within a study site. Within-site mortality was inferred from a prolonged cessation of movements, and was confirmed in spring during the final tracking survey, by disturbing the substrate to determine if the tagged parr was still alive. In addition, stream banks were searched for tags in spring to document additional mortalities.

The population used for the apparent survival analysis was defined as the number of tagged salmon parr found at each study site during the first active tracking survey, and therefore, tagged salmon parr that were never located within

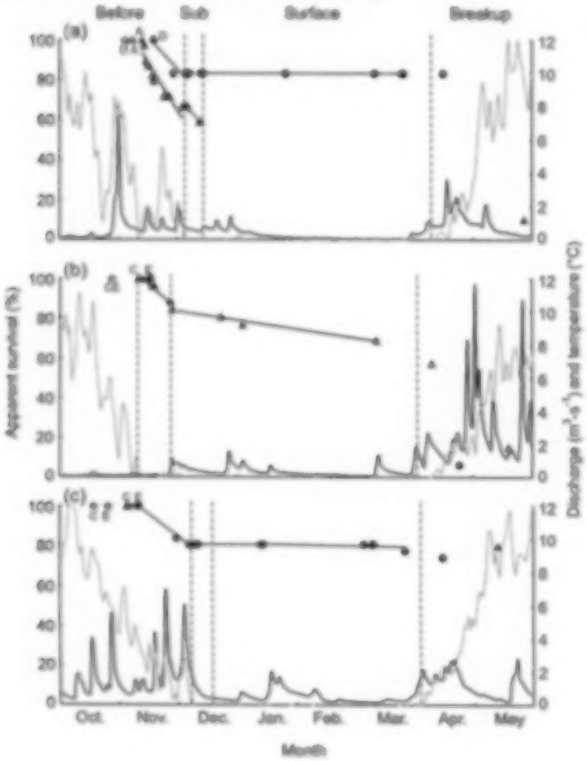

Fig. 2. Stream discharge (black line), water temperature (gray line), tagging dates (°) in different sites of Catamaran Brook, and apparent survival of Atlantic salmon parr during different periods of the three winters: (*a*) = 2003–2004, (*b*) = 2004–2005, (*c*) = 2005–2006. Triangles and circles represent the data collected from Lower and Middle Reach, respectively, and the letter above the symbol refers to the study site as described in Table 2. Dashed vertical lines represent the different periods of winter ("Before" = before ice; "Sub" = subsurface ice; "Surface" = surface ice; "Breakup" = after ice breakup). Data points within the same period are connected with a simple linear regression line to make the interpretation of the survival pattern more comprehensive. The stream discharge is shown for the site D. Note that no before-ice data is shown in panel B because of early freeze-up.

study sites were omitted from the subsequent analysis of apparent survival (see Results). This was necessary as a precautionary approach because of the pervasive capture method (i.e., electrofishing) and tagging procedure may have artificially increased emigration initially (Nordwall 1999; Brakensiek and Hankin 2007). Handling-related emigration has been shown to mostly occur within 24 h from PIT tagging (Enders et al. 2007). Tagging-related mortality mostly occurs up to 3–5 days after PIT tagging (Sigourney et al. 2005; Bateman and Gresswell 2006), and the majority of displaced fish settle in new areas in less than 3 days (Armstrong et al. 1997). For these reasons, any tagging-induced bias in apparent survival calculations should have been minimized, as the first active tracking of fish followed only after 7–23 days after tagging.

Mean values throughout this article are reported with the corresponding standard error (SE). If multiple group means were compared, logarithmic transformations were used to normalize the data if deemed necessary. The effect of year (Y) was used as a random variable and reach (R) as a fixed factor in analyses. In comparisons examining the parr status (S) as being present or absent, the status was used as a fixed factor in the analyses. Post hoc comparisons were carried out using applicable Tukey-type tests (Zar 1999), and arcsine transformation was applied if post hoc comparisons involved proportions. Data collected from site D were excluded from the statistical analysis for apparent survival because of low sample size ($n = 6$). Continuous data collection from sites E (2004–2005) and C (2005–2006) was not possible, and therefore the apparent survival during each period of winter could not be assessed (i.e., data was limited to autumn–spring survival data). The propensity of emigration for mature versus nonmature parr was examined using odds ratios (Sokal and Rohlf 1995). The odds ratios were tested for homogeneity across the study sites and years using Tarone's χ^2 test; since the odds ratios were considered homogenous ($\chi^2 = 2.7$, df = 5, $P > 0.05$), the subsequent significance of overall average odds ratio was based on the Mantel–Haenszel estimate o_{MH} (Sokal and Rohlf 1995). When the exact timing of emigration was known from stationary antennae, its relationship to discharge (Q) and water temperature (T) was examined using logistic regression. The goodness-of-fit of the models was based on the Hosmer–Lemeshow (χ^2_{HL}) test. The relative fit of each of the a priori candidate models was assessed using Akaike's information criterion (AIC_c) values adjusted for small sample bias (Burnham and Anderson 2002). The relative plausibility of each model was further based on Akaike weights, w_i, so that the most plausible model (i.e., Kullback–Leibler best model; K-L) had the largest weight (Burnham and Anderson 2002). The 95% confidence set of the actual K-L best model was defined as a group of models, arranged by their Akaike weight from largest to smallest, for which the sum of Akaike weights exceeded 0.95. The relative importance of each predictor variable was assessed by summing the w_i values across the models in the confidence set where each variable occurred and was considered significant based on Wald's measure. The statistical analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, Illinois).

Results

Apparent survival and observed mortality

Population dynamics before first active tracking survey

The initial decline in the number of tagged salmon parr during the period between tagging and the first active tracking survey was relatively high in all 3 years (Table 5). The decline was similar between the two stream reaches in each year (Fisher exact, $P > 0.05$ for all years). However, interannual differences in the proportionate decline were observed in the Lower Reach, but not in the Middle Reach (Table 5; Lower Reach, $G = 9.37$, df = 2, $P < 0.01$; Middle Reach, $G = 3.84$, df = 2, $P > 0.05$). A nonquantitative analysis suggested a relationship between high stream discharge fluctuations and the intensity in decline in the number of salmon

Table 4. Number of separate dates during different periods of winter when active tracking surveys were carried out in each study site of Catamaran Brook, New Brunswick, and the total number of active tracking surveys carried out at each site.

	No. of tracking dates at each study site (year)					
Tracking period	A (2003–2004)	C (2004–2005)	C (2005–2006)	D (2003–2004)	E (2004–2005)	E (2005–2006)
Before ice	10	1	2	2	0	3
Subsurface ice	5	4	0	4	2	4
Surface ice	0	0	0	5	0	6
After breakup	1	2	1	1	1	2
Total trackings*	34	16	9	27	10	47

*Tracking of tagged fish occurred multiple times within most dates; these data are considered elsewhere (Linnansaari 2009).

Table 5. The percentage of absent tagged Atlantic salmon parr before first active tracking survey in the two reaches of Catamaran Brook.

	2003–2004	2004–2005	2005–2006
Lower Reach	40.4ab (57)	27.8a (36)	64.5b (31)
Middle Reach	57.1a (28)	30.4a (23)	41.5a (53)

Note: Numbers in parentheses refer to the number of salmon parr initially tagged each year. The values sharing the same letter within each row were not significantly different from each other (Tukey-type post hoc test using arcsine-transformed data; $P > 0.05$).

parr. The lowest decline in both reaches occurred during the winter of 2004–2005, which was a year with stable, low discharge during the time period from tagging to the first tracking survey (Fig. 2). The highest decline in the number of tagged salmon parr in the Lower Reach was observed in 2005–2006 when a high discharge event occurred between the tagging and first tracking date (Fig. 2), whereas the highest decline in the Middle Reach occurred in 2003–2004 when the tagged salmon parr were released during relatively high stream flows immediately following a high discharge event (Fig. 2).

The decline in the number of tagged salmon parr before the first tracking survey also depended on precocious maturity. The likelihood of mature parr being absent from the study area before the first tracking survey was 2.8 times that of immature parr ($\omega_{MH} = 2.81$, $\chi^2_{MH} = 8.04$, df = 1, $P < 0.01$). However, there was no significant difference between absent and resident parr with respect to size (L_F or mass), condition factor (Fulton's K; Ricker 1975) or W_T/W_F ratio (three-factor$_{(Y,R,S)}$ analysis of variance (ANOVA), all main effects and interaction terms for all four variables $P > 0.05$).

Population dynamics during the monitored period

Apparent survival was lowest during the before-ice period in autumn, and the decline in the number of tagged salmon parr ranged from 16.7% to 33.3% (26.3% for data pooled across sites and years; Table 6, Fig. 2). Apparent survival was better during both subsurface and surface ice periods, the decline in the number of tagged salmon parr ranging from 0% to 15.4% (pooled data across sites and years; 6.9% and 10.0%, respectively; Table 6, Fig. 2). Statistically, the decline in the number of tagged Atlantic salmon parr was significantly higher during before-ice than subsurface ice period (Fisher's exact test with Bonferroni adjusted P; site A, 2003–2004, $P = 0.020$; site E, 2005–2006, $P = 0.048$), whereas it was similar between subsurface and surface ice periods (site C, 2004–2005, $P = 1.000$; site E, 2005–2006, $P = 0.480$). In total, the tagged salmon parr population declined 16.7%–42.3% over winter (Table 6), but the apparent survival was statistically similar between years ($G = 2.51$, df = 2, $P = 0.286$); estimated total decline over winter was 31.7% for the pooled data (i.e., apparent survival of 68.3%) considering the study sites that could be assessed soon after ice breakup.

The mean instantaneous daily loss rate was highest during the before-ice period (Table 6). The fact that the period with surface ice lasted longer than the other winter periods in Catamaran Brook in each study year (Fig. 2) contributed to the low mean instantaneous daily loss rate during surface ice period (Table 6).

The observed within-site mortality during winter was low (pooled data 2003–2006; $n = 8$, 4.4% of the parr observed at least once). On occasion the mortality could be related to predation (i.e., tags recovered from the stream banks, $n = 3$) that occurred before ice cover formed. Of the remaining known mortalities, four took place during surface ice or spring period for unknown reasons, and the timing or the reason for one mortality could not be determined.

Factors affecting apparent survival and observed emigration

The size of the tagged salmon parr in autumn did not affect the probability of remaining within a study site over winter. No statistical differences were observed in L_F, mass, K, or W_T/W_F ratio between the parr that disappeared over winter and those that remained resident within study sites until spring (three-factor$_{(Y,R,S)}$ ANOVA, $P > 0.05$ for all main effects and interaction terms for all four variables). Occasionally, emigration (or involuntary displacement) was influenced by localized "catastrophic events". An example of this was a release and redistribution of a logjam in November 2005 that lead to a mesohabitat shift in site E; a section of 20 m^2 of slow flowing glide habitat was transformed into fast flowing riffle. During the logjam release, all tagged fish ($n = 3$) in the affected area emigrated, whereas no emigration occurred from other parts of the study site regardless of the relatively high discharge during the event.

Stream discharge (Q) proved to be a much more important predictor to estimate the probability of juvenile salmon emigration in Catamaran Brook in winter than stream temperature (T) or their interaction $Q \times T$ (relative variable importance 0.87 for Q, 0.08 for $Q \times T$, and 0.0 for T). All except one of the supported logistic regression models included discharge as a plausible predictor (Table 7). How-

Table 6. Proportional decline of PIT-tagged Atlantic salmon parr population (%) and the instantaneous daily loss rates (in parentheses) in each study site in Catamaran Brook during different periods of winter, with the mean (and standard error, SE) instantaneous daily loss rate (calculated across the study sites) within each period of winter.

	Study site (year)				
	A (2003–2004) [n = 39]	D (2003–2004)* [n = 6]	C (2004–2005) [n = 26]	E (2005–2006) [n = 31]	Mean (SE) loss rate
Before ice	33.3 (0.019)	16.7 (0.011)	NA	19.4 (0.008)	0.014 (0.006)
Subsurface ice	7.7 (0.014)	0.0 (0.000)	15.4 (0.010)	0.0 (0.000)	0.008 (0.004)
Surface ice	NA	0.0 (0.000)	15.4 (0.002)	3.2 (0.000)	0.001 (0.001)
Total winter decline	NA	16.7 (0.001)	42.3 (0.004)	25.8 (0.002)	0.002 (0.001)

Note: Values in brackets (*n*) represent number of tagged parr during first tracking survey; NA, data not available. Resulting total decline of PIT-tagged Atlantic salmon parr population at the end of winter is shown.

*The values from site D are not used to calculate the mean because of the low number of observations.

ever, goodness-of-fit was not very strong for any of the models (P for χ^2_{HL} ranging from 0.06 to 0.36), and there was a large amount of unexplained variability in all models (Nagelkerke pseudo-r^2 ranged from 0.18 to 0.23).

The response of salmon parr to two midwinter moderate discharge events (relative to winter base flow) was analyzed in some detail. The first midwinter flood started 25 December 2004; the second midwinter ice breakup and flood started 15 January 2006; Fig. 2). Based on stationary antenna data, only one tagged salmon parr emigrated from site C during the 2004 event (7 days after the peak flood). No changes in apparent survival were observed during the 2006 event based on active tracking on 19 January 2006 in parts of study site E that could safely be tracked (~35% of the site).

The extent of emigration beyond study site E was assessed in the spring of 2006 with the two-person antenna. Of the tagged salmon parr that were not encountered within study site E after ice breakup, a significantly higher proportion of the immature parr (73%) were found compared with mature parr (9%; Fisher's exact test; $P = 0.008$). Every parr found beyond the study site ($n = 9$) was alive. Furthest fish were found 285 m upstream and 290 m downstream from the study site. Based on the stationary antennae data in the autumn 2003–2004 and winter 2004–2005, it was apparent that longer distance emigration (i.e., >5 km) occurred, as a few parr tagged in the Middle Reach were observed in the Lower Reach emigrating out from Catamaran Brook during autumn ($n = 1$) and midwinter ($n = 2$). All these three parr were of a size that potentially smoltified in spring (L_F: 107–119 mm).

Discussion

The apparent survival of Atlantic salmon parr was not static throughout the winter in Catamaran Brook. Lowest apparent survival was observed in late autumn – early winter before ice formed and coincided with a rapid decline in water temperature and fluctuation in stream discharge. Apparent survival showed more variability during the period dominated by subsurface ice and further improved when surface ice was prevailing. Actual (observed) mortality within the study sites was low during all three winters. Although winter, as an entity, is sometimes referred to as a critical period for survival for stream salmonids (Cunjak and Therrien 1998; Bradford et al. 2001), the current study emphasized the fact that higher temporal resolution may be necessary to be able to correctly predict possible consequences of anthropogenic impacts in future. For example, if winter is considered as a "bottleneck" for juvenile stream salmonids, the current scenarios on global warming that predict shorter periods with ice and snow in the future for all of Europe and most of North America (Christensen et al. 2007) may be seen as beneficial for salmonid stocks. However, the current study showed that shortening of the ice-affected period of winter would not positively affect apparent survival of juvenile Atlantic salmon. Furthermore, it is possible that the ice-covered period of the year will be replaced with conditions similar to those observed during late autumn – early winter (Prowse and Beltaos 2002), a change that would very likely cause repercussions in terms of declining apparent survival of juvenile Atlantic salmon.

The current study is by no means the first one to report a decline in apparent survival in juvenile stream salmonid populations in late autumn – early winter. Autumnal decline in density of juvenile salmonids seems a widely observed phenomenon (Youngson et al. 1983; Carlson and Letcher 2003; Linnansaari et al. 2009). Also the "winter decline" data in Letcher et al. (2002) shows the steepest decline in the estimated apparent survival of Atlantic salmon during before-ice conditions in autumn in West Brook, Massachusetts, USA. To our knowledge, however, this is the first study where sampling (i.e., tracking) was frequent enough to accurately distinguish and quantify the relative severity of different periods of winter in terms of apparent survival of tagged salmon parr in an ice-covered natural stream. This was also the first to document that the autumnal before-ice period may be the time of the most severe decline in apparent survival.

In Catamaran Brook, many reasons could have contributed to the observed decline in apparent survival in autumn. First, precocious maturity was strongly associated with increased emigration from the study sites coincident with the approaching spawning period. Maturity-related autumn dis-

Table 7. The comparison of logistic regression models for predicting the probability of emigration (P_{emig}) of salmon parr and the parameter estimates ± standard error (SE) for each supported model.

Model	AIC_c	w_i	Evidence ratio	Q	T	$Q \times T$	α
$Z_{(Q\alpha)}$*	96.9	0.348	1	1.29±0.34	—	—	−3.02±0.49
$Z_{(Q,Q\times T,\alpha)}$*	97.7	0.228	1.5	0.94±0.46	—	0.15±0.14	−2.85±0.50
$Z_{(Q,T,\alpha)}$*	97.9	0.215	1.6	1.06±0.40	0.26±0.25	—	−2.97±0.49
$Z_{(Q,T,Q\times T,\alpha)}$*	99.8	0.081	4.3	0.96±0.48	0.06±0.61	0.12±0.34	−2.88±0.55
$Z_{(Q\times T,\alpha)}$*	99.8	0.081	4.3	—	—	0.38±0.11	−2.14±0.31
$Z_{(T,Q\times T,\alpha)}$	101.6	0.033	10.6				
$Z_{(T,\alpha)}$	103.3	0.014	24.3				
$Z_{(\alpha)}$	111.9	0.000	1854.6				

Note: AIC_c, Akaike's information criterion corrected for small samples; w_i, Akaike weights; Q, stream discharge; T, water temperature; α, estimated constant for each model. Interpretation of w_i is considered as the relative plausibility of candidate models whereas evidence ratio describes the likelihood of the best model being correct over the model in comparison. $P_{emig} = 1/[1 + e^{-Z}]$, where $Z = \alpha + \beta_1 X_1 + \ldots + \beta_i X_i$, and $X_1 \ldots_i$ are fitted variables, and α and $\beta_1 \ldots_i$ are estimated parameters. Significant variables (Wald's measure, $P < 0.05$) in each model are shown underlined.

*Models within the confidence set.

persal has also been observed in other studies (e.g., Buck and Youngson 1982; Whalen et al. 1999; Linnansaari et al. 2009). Second, the large interannual variability in the estimates of early winter apparent survival suggested that environmental factors might be controlling the survival pattern. In particular, stream discharge seemed to partly explain the phenomenon in Catamaran Brook. In addition to the evidence from the stationary antennae and the logistic regression models where discharge was supported as an important variable, the apparent survival following tagging was highest during the year with the most stable stream discharge, whereas largest amount of tagged salmon parr were absent when the release of the fish coincided with high flow events. However, no threshold size for a discharge event triggering emigration could be established. Certainly, the moderate discharge events in midwinter did not cause decline in apparent survival. Therefore, it seemed that the lowered apparent survival was linked to frequent fluctuation of flow rather than the absolute magnitude of discharge. Earlier research has shown that early winter period may be energetically demanding for juvenile salmonids, and acclimatization to changing conditions may lead to metabolic deficiencies (Cunjak and Power 1987; Cunjak et al. 1987; Cunjak 1988). The interplay between possible development of metabolic deficiency and lower apparent survival during acclimatization period in early winter warrants further research.

Production of subsurface ice (frazil ice, anchor ice) did not cause drastic decline in apparent survival in Catamaran Brook during any of the three study winters. Although some emigration was observed, the apparent survival was better than during the before-ice period for all studied years. Moreover, no within-site mortality was observed during the subsurface ice period, and it seems that mortality by means of frazil crystals clogging the mouth and gills of fish (Tack 1938; see also Brown et al. 1993) may be a relatively rare phenomenon. Many studies have reported immediate escape responses (Brown and Mackay 1995), large-scale emigration or redistribution (Jakober et al. 1998; Whalen et al. 1999), habitat exclusion (Maciolek and Needham 1952; Stickler et al. 2007), and even mortality (Bradford et al. 2001) for various salmonid populations once subsurface ice conditions occur; hence, such conditions have been considered to be unsuitable for stream salmonids. By contrast, Roussel et al. (2004), working also in Catamaran Brook, observed high site fidelity during anchor ice events especially in riffle habitats, but the observations were based on a small sample size. In the present study, we followed 83 individuals during multiple, very severe anchor ice events, and our results corroborate those of Roussel et al. (2004). It was evident that the relatively small-sized juvenile salmon that we tracked in the current study were able to find adequate shelter within the interstices of coarse substrate material in Catamaran Brook. Salmon parr are able to bury themselves under 15–20 cm layer of stones (Erkinaro et al. 1994) where the (hyporheic) water temperature is elevated to a level where subsurface ice does not occur (i.e., >1 °C; Caissie and Giberson 2003). Heggenes et al. (1993) suggested that large-sized salmonids (i.e., >25 cm) may not be able to find suitable interstitial cover and therefore adopt different behavioural tactics in winter, including aggregation into pool habitats or emigration. Thus, the contrasting response of salmon parr to subsurface ice between this and some of the former studies (Brown and Mackay 1995; Jakober et al. 1998; Brown 1999) may be a consequence of differences in body size (mean of 8.9–11.5 cm in the current study vs. >25 cm in above mentioned studies) and the consequent ability to find suitable cover during subsurface ice formation. However, the reasons for interannual variability in the proportionate decline in fish numbers during subsurface ice period warrants more research and may be caused by the physical characteristics of anchor ice (Stickler and Alfredsen 2009).

Apparent survival was the highest during the period dominated by surface ice, and the instantaneous loss rate during the period with surface ice was similar to the rate in a regulated ice-covered stream in Norway (0.002 vs. 0.001 in the current study; Linnansaari et al. 2009). Although midwinter is reported to be a period of the year that may limit the production of Atlantic salmon parr because of a low minimum discharge and subsequent limitation in the amount of suitable rearing habitat (Gibson and Myers 1988; Hvidsten 1993), it seemed that the stability of environmental conditions during the ice-covered period of winter overruled possible drawbacks. Loss of wetted area was minimal in the current study, and tagged Atlantic salmon parr were able to

remain very close to their original locations throughout the winter. Moreover, the relatively low density of conspecifics (that might compete for available shelters) further facilitated finding a new location in close proximity of the original location if the original areas became unsuitable (e.g., dewatered). Simulated surface ice cover has been shown to positively affect the energy budget of salmon parr (Finstad et al. 2004*b*), which indicates that high fidelity can be expected during midwinter, although rarely documented in experiments in situ. Nevertheless, it has to be kept in mind that energy reserves of juvenile salmonids tend to decrease throughout the winter (Cunjak 1988; Næsje et al. 2006), and the effects (e.g., "death toll") of wintertime carry-on metabolic decline may be realized only after the conditions require higher energetic expenditure, like the period during and immediately after ice breakup. Such speculation is supported by observations from Norway where Finstad et al. (2004*a*) detected greater mortality of Atlantic salmon juveniles with low energy reserves on two different occasions during a 3-year study; both occasions occurred late winter – spring coincident with temperature–discharge fluctuation.

Overall, the estimate of apparent survival over the whole winter in this study fell within the wide range of survival estimates previously reported for juvenile stream salmonids (Huusko et al. 2007). However, low (actual) within-site mortality throughout the three studied winters and the fact that majority of the emigrant immature parr were re-encountered alive in the two-person antenna survey extended beyond the study site E in the Middle Reach in the spring of 2006 lead us believe that the decreasing number of tagged salmon parr in the studied sites was, at least initially, mainly due to emigration rather than mortality. Regardless of the fact whether the decline of tagged salmon parr was due to actual mortality, it appears that substantial decline in apparent survival of salmon parr must occur also during other seasons than winter in Catamaran Brook, as the annual survival estimates have been previously reported to be only 12.9% for comparable age group (Cunjak and Therrien 1998). Indeed, recent review articles on overwinter survival of stream-dwelling juvenile salmonids do not support the concept that winter should be automatically considered as a specific bottleneck period for survival in temperate areas (Huusko et al. 2007; Carlson et al. 2008).

In conclusion, our data suggest that while a definite decline in apparent survival occurred in winter in Catamaran Brook, the apparent survival of Atlantic salmon parr may not be static throughout the winter in streams where different physical (ice) conditions are experienced. The decline in apparent survival in the current study was most pronounced prior to ice formation but was relatively high and stable especially during the period affected by surface ice. With increasing anthropogenic impacts affecting fluvial biota, it is of crucial importance to continue to study the population dynamics of juvenile salmonids at the subperiod scale during winter in the future.

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Predicting the vertical distributions of reef fish larvae in the Straits of Florida from environmental factors

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Abstract: Three seasons of vertically stratified ichthyoplankton sampling at the edge of the Florida Current revealed consistent accumulations of some coral reef fish larvae under taxon-specific environmental conditions. Environmental variability ranging from predictable (seasonal differences in temperature, diel changes in light, and vertical gradients in many variables) to stochastic (changes in wind-driven turbulence and patchiness of zooplankton) was used to model larval distributions. In five taxa, including the commercially important Epinephelini (groupers), relative larval densities were predicted with significant accuracy based entirely on sampling depth. Models yielding these predictions were cross-validated among all seasons, indicating that larval vertical distributions were remarkably unaffected by other environmental factors, while revealing strong behavioral preferences for specific ranges of hydrostatic pressure. Pomacentridae (damselfish) larvae consistently occupied shallower depths at night than during the day, demonstrating diel vertical migrations. At the community level, depth and season were two major factors structuring larval coral reef fish assemblages. Predictable vertical distributions of larvae in the Straits of Florida can facilitate modeling the same taxa elsewhere in the Western Central Atlantic.

Résumé : Un échantillonnage stratifié verticalement de l'ichtyoplancton sur trois saisons à la marge du courant de Floride montre des accumulations régulières de larves de certaines espèces de poissons de récifs coralliens dans des conditions environnementales spécifiques à chaque taxon. Nous avons utilisé la variabilité environnementale allant de prévisible (différences saisonnières de température, changements diurnes de lumière et gradients verticaux de plusieurs variables) à stochastique (changements dans la turbulence sous l'effet du vent et répartition en taches du zooplancton) pour modéliser les répartitions des larves. Chez cinq des taxons, y compris les Epinephelini (les mérous) d'importance économique, la profondeur d'échantillonnage seule permet de prédire les densités relatives des larves avec une forte précision. Les modèles qui fournissent ces prédictions ont été soumis à une validation croisée sur toutes les saisons, ce qui indique que les répartitions verticales des larves sont remarquablement peu affectées par les autres facteurs du milieu et que les larves manifestent de fortes préférences comportementales pour des gammes spécifiques de pression hydrostatique. Les larves de Pomacentridae (demoiselles) se retrouvent de façon constante à des profondeurs moins grandes la nuit que le jour, ce qui démontre l'existence de migrations verticales diurnes. Au niveau de la communauté, la profondeur et la saison sont deux facteurs dominants dans la structuration des peuplements de larves de poissons de récifs coralliens. La répartition verticale prévisible des larves dans le détroit de Floride peut faciliter la modélisation des mêmes taxons ailleurs dans le centre de l'Atlantique de l'Ouest.

[Traduit par la Rédaction]

Introduction

Marine fish larvae, like other zooplankton, rarely distribute randomly in the ocean water column. Instead, they assume distinct vertical distributions depending on the species and environment (reviewed in Heath 1992). Because the environmental conditions larvae experience vary considerably among different depths, vertical distributions can affect essential ecological processes such as feeding, transport, growth, and survival. Vertical gradients in physical factors such as visible light (Forward et al. 1996), ultraviolet (UV) radiation (Browman 2003), pressure (Huebert 2008), turbulence (Werner et al. 2001), temperature (Olla et al. 1996), and salinity (Lougee et al. 2002) are thought to influence larval distributions. Vertical patterns of predators and prey are also considered important (Fortier and Leggett 1983; Fortier and Harris 1989). Some fish larvae actively seek out or avoid specific environmental conditions by vertical swimming (Olla et al. 1996; Huebert 2008), and those with physoclistic swimbladders can remain in a preferred environment

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Table 1. Sample sizes of coral reef fish larvae collected in spring, summer, and fall MOCNESS (Multiple Opening Closing Net with Environmental Sampling System) tows at a fixed station in the Straits of Florida over two diel cycles from 0 to 100 m depth.

Taxon	Spring	Summer	Fall
Acanthuridae	9	**45**	**129**
Apogonidae	**118**	10	**35**
Aulostomidae	—	—	2
Balistidae	41	23	17
Suborder Blennioidei	35	1	—
Callionymidae	14	20	49
Carapidae	12	15	41
Chaetodontidae	6	4	12
Cirrhitidae	—	—	9
Dactylopteridae	—	3	—
Diodontidae	—	6	—
Fistularidae	1	—	1
Gerreidae	2	1	11
Suborder Gobioidei	**89**	**64**	**223**
Haemulidae	3	3	20
Holocentridae	**131**	**80**	**47**
Kyphosidae	—	2	4
Labridae[a]			
Clepticus spp.	2	1	8
Decodon puellaris	5	4	24
Doratonotus megalepis	1	1	18
Halichoeres spp.	11	6	9
Lachnolaimus maximus	4	—	—
Thalassoma bifasciatum	3	12	105
Xyrichtys spp.	1	25	265
Lutjanidae			
Subfamily Etelinae			
Etelis oculatus	1	1	48
***Pristipomoides* spp.**	1	**145**	**225**
Subfamily Lutjaninae			
Rhomboplites aurorubens	10	—	23
Other	9	25	34
Monacanthidae	76	15	18
Mullidae[b]	103	3	2
Opistognathidae	4	—	5
Ostraciidae	2	4	6
Pomacanthidae	—	27	22
Pomacentridae			
Abudefduf spp.	3	3	1
Chromis spp.	1	0	4
Other	**44**	**47**	**75**
Priacanthidae	**83**	**121**	**57**
Scaridae			
Cryptotomus roseus	5	9	32
Scarus spp.	1	1	4
***Sparisoma* spp.**	**31**	**47**	**171**
Scorpaenidae	**113**	**145**	**1014**
Serranidae			
Subfamily Anthiinae			
Anthias nicholsi	138	4	29
Anthias tenuis	5	—	—
Anthias woodsi	19	2	7
Hemanthias leptus	351	18	17
Hemanthias vivanus	**2070**	**37**	**62**
Plectranthias garruppellus	—	—	2
Pronotogrammus martinicensis	—	—	3
Unknown	7	—	—
Subfamily Epinephelinae			
Tribe Epinephelini	**33**	**30**	**242**
Tribe Grammistini	1	14	18
Tribe Liopropomini	—	10	52
Subfamily Serraninae	**154**	**33**	**115**
Spuridae	17	—	1
Sphyraenidae	**33**	**76**	**80**
Syngnathidae	13	3	3
Synodontidae	16	1	59
Tetraodontidae[b]	41	14	35

Note: Bold type indicates taxa included in vertical distribution models because of sample sizes of ≥30 in at least two seasons. Total volume sampled was highest in Fall (100%), lowest in summer (82%), and intermediate in spring (88%).

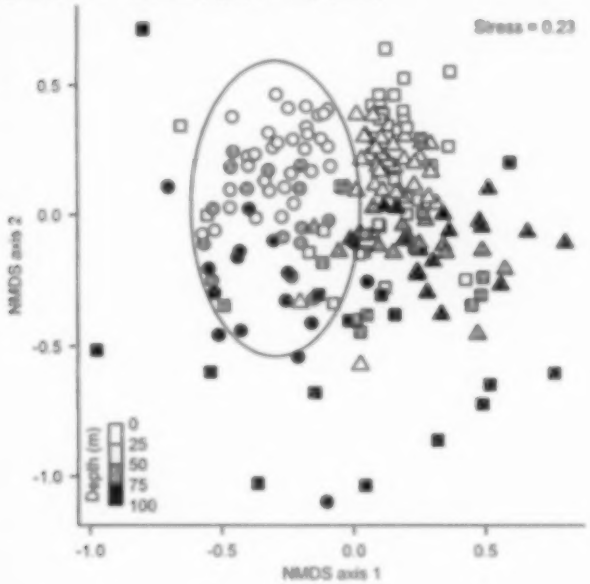
[a]Labridae were not pooled because different genera exhibited dissimilar vertical distributions.

[b]Mullidae and Tetraodontidae were caught in larger numbers in neuston net tows at <0.5 m depth than in MOCNESS tows and were excluded from analysis.

without expending the energy to swim by actively maintaining neutral buoyancy (Govoni and Hoss 2001). Coral reef fish larvae have physoclistic swimbladders and are fast swimmers relative to other fish larvae (Leis 2006), thus they may exercise a particularly high degree of control over their vertical distributions.

Previous studies of reef fish larvae have found significant differences in vertical distributions between day and night (Leis 1986, 1991*b*) and between early and late stage larvae (Cowen 2002), revealing the dynamic nature of vertical patterns. However, the role of specific environmental variables in influencing empirical larval reef fish distributions has not been examined quantitatively. The goals of the present study were to (*i*) measure the vertical distributions of coral reef fish larvae under a wide range of environmental conditions; (*ii*) develop statistical models that predict larval distributions based on environmental data; (*iii*) test the accuracy of model predictions; and (*iv*) use the accuracy of model predictions to estimate the importance of environmental factors in shaping larval vertical distributions. The focal data set of ichthyoplankton samples from the Straits of Florida represents one of the most extensive vertically stratified collections of reef fish larvae sampled at high temporal resolution. The analysis provides a novel quantitative approach to describe the influence of environmental conditions on larval fish distributions.

Fig. 1. Two-dimensional ordination of ichthyoplankton samples, using Kruskal's nonmetric multidimensional scaling of Bray–Curtis dissimilarity with respect to taxonomic composition across 30 coral reef fish families and the suborders Blennioidei and Gobioidei. Fish larvae were collected in the Straits of Florida during three seasons at four depth ranges. Two strong patterns are apparent: First, samples collected during spring (circles) cluster separately from summer (squares) and fall (triangles) samples, as illustrated by the ellipse. Second, samples collected at different depths form a distinct gradient from shallow (light) to deep (dark).



Materials and methods

Sampling

To characterize the vertical distributions of fish larvae in the Straits of Florida across a wide range of environmental conditions, three time series of biological and physical measurements were collected in spring, summer, and fall 2003. All time series involved repeated sampling of the water column every 3 h for two diel cycles. Sampling was conducted from the University of Miami's RV *F.G. Walton Smith*. During the spring time series from 7 April to 9 April and the fall time series from 30 September to 2 October, the vessel maintained position at a station ~30 km SSE of Miami (25.5°N, 80.06°W) with a bottom depth of ~130 m for 48 h of sampling. During the summer time series from 31 July to 2 August, the vessel maintained position at an adjacent station (25.5°N, 80.05°W) ~1 km farther offshore with a bottom depth of ~160 m for 42 h of sampling. Over the course of each time series, different parcels of water reflecting a variety of environmental conditions were transported past the sampling station by the Florida Current.

Ichthyoplankton samples were collected by towing a coupled asymmetrical Multiple Opening Closing Net with Environmental Sampling System (MOCNESS) (Guigand et al. 2005) obliquely from 100 m depth to the surface at a tow speed of 1.5 m·s^{-1}. Two parallel sets of nets, one with 1 m^2 mouth opening and 150 μm mesh and one with 4 m^2 mouth opening and 1 mm mesh, were opened and closed such that a different pair sampled from 100–75, 75–50, 50–25, and 25–0 m. The volume sampled by each net was calculated from flow through the net (MOCNESS flowmeter) and the mouth opening of the net corrected by its angle of attack (MOCNESS frame angle sensor). Additional sampling of the upper 0.5 m of the water column was conducted by towing adjoined neuston nets (0.5 m^2 with 150 μm mesh, 2 m^2 with 1 mm mesh) following each MOCNESS tow. Plankton samples were immediately fixed in 95% ethanol and later transferred to 70% ethanol for long-term storage. Larval fishes from only the 1 mm mesh nets were identified to family following Richards (2006). Larvae from the most abundant coral reef fish families were then further identified to the lowest possible taxonomic level. The settled plankton volume in samples from 150 μm mesh nets was used as a proxy for zooplankton biomass.

Physical measurements recorded by the MOCNESS included pressure, temperature, salinity, chlorophyll fluorescence (in summer and fall), and light (downwelling photosynthetically active radiation; in fall). Following each MOCNESS tow, a vertical profile of depth, salinity, temperature, fluorometry, oxygen saturation, and light transmission was collected with a CTD (conductivity–temperature–depth) instrument. In spring and summer, light-at-depth was calculated from shipboard radiometer and CTD transmissometer measurements. Light measurements were log-transformed. For all variables, the measurement taken closest to the midpoint of each sampled depth range, which generally corresponded to the median value (R.K. Cowen, unpublished data), was used to characterize the sample. Hourly wind speeds were obtained from the NOAA Fowey Rocks weather station, located 11 km to the northwest of the sampling station. Water level measurements at the NOAA Vir-

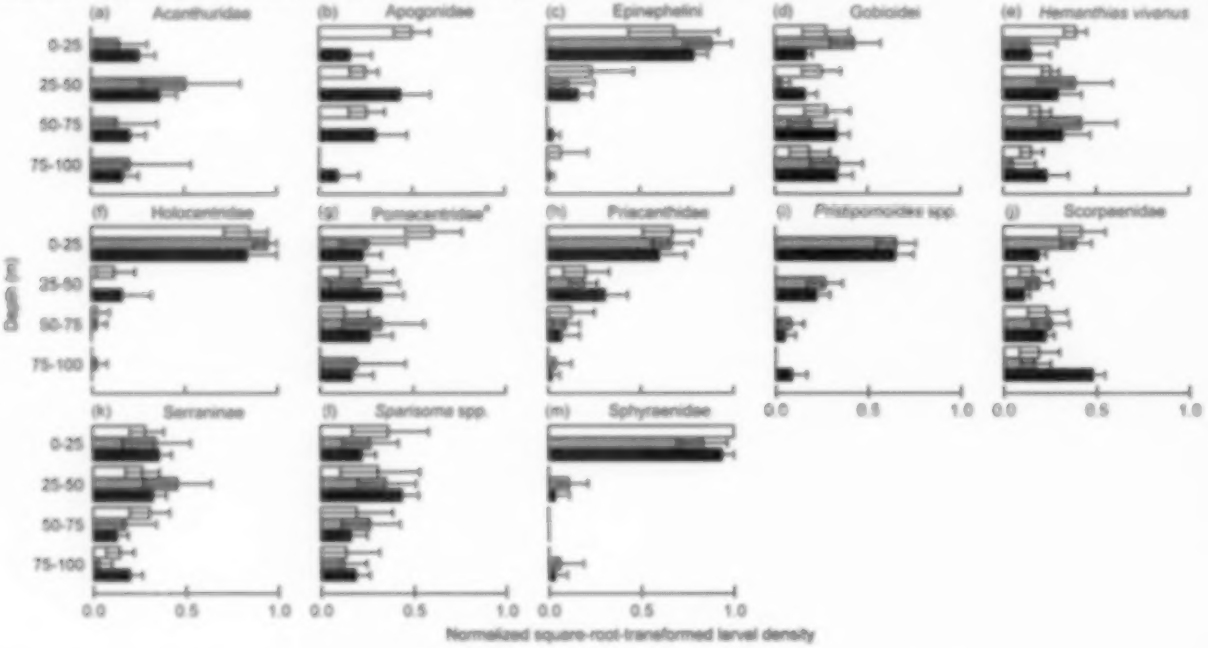
Table 2. Diel variability in densities of common coral reef fish larvae collected in the Straits of Florida at 0–100 m depth in three seasonal 42–48 h duration time series of MOCNESS tows, expressed as percentages collected during the daytime (missing values (—) indicate sample sizes <30).

Taxon	Spring	Summer	Fall
Acanthuridae	—	51	52
Apogonidae	62	—	70
Epinephelini	46	41	42
Gobioidei	46	44	45
Hemanthias vivanus	41	38	73
Holocentridae	33	43	53
Pomacentridae[a]	51	52	51
Priacanthidae	41	48	62
Pristipomoides spp.	—	41	48
Scorpaenid	45	61	29
Serraninae	38	47	46
Sparisoma spp.	**15**	**9**	**20**
Sphyraenidae	67	36	62

Note: <20% of *Sparisoma* spp. larvae (bold) were collected during the day and >80% during the night, revealing significant diel net avoidance (permutation test: $p < 0.05$).

[a]Excluding the genera *Abudefduf* and *Chromis*.

Fig. 2. Vertical distributions of larvae from 13 common coral reef fish taxa sampled at four different depth ranges in the Straits of Florida during three 42–48 h time series in spring (open), summer (grey), and fall (black) 2003. Error bars represent bootstrapped 95% confidence intervals. [a]In panel (*g*) for Pomacentridae, data exclude the genera *Abudefduf* and *Chromis*.



ginia Key station, 28 km to the northwest of the sampling station, were used as a tidal phase index. Mixed layer depth was defined as the depth above which no gradient in CTD temperature or salinity measurements was apparent. Under this definition, mixed layer depth captured the effects of recent turbulent mixing and was independent from and generally much shallower than the main (seasonal) pycnocline.

Data analyses

Data analyses were focused on identifying ecologically meaningful and statistically robust relationships between vertical distributions of larvae and their environment. Given the large number of potentially correlated variables and the uncertain degree of statistical independence among larvae from the same net, adjacent nets from the same tow, and different tows from the same cruise, hypothesis testing required particular care. To reduce the chance of type I errors (incorrectly rejecting null hypotheses), associations between variables were only considered significant if they could be cross-validated (i.e., if consistent patterns were present in all analyzed sampling periods). Cross-validation also provided a safeguard against the misinterpretation of horizontal patterns (patchiness among larvae drifting past the station) as vertical patterns.

For each coral reef fish taxon, time series with sample sizes <30 larvae were considered insufficient for analysis and excluded. Further, cross-validation required analysis of at least two sampling periods; thus, taxa with sample sizes <30 in two time series were excluded entirely. Pomacentridae, except for the genera *Abudefduf* and *Chromis*, (hereafter referred to as pomacentrids) were pooled at the family level. Labridae were excluded because the vertical distributions of different genera ranged from very shallow (*Lachnolaimus maximus*, *Doratonotus megalepis*) to very deep (*Decadon puellaris*), and no single genus was present in sufficient numbers. Tetraodontidae were excluded because MOCNESS samples alone were not considered representative of the population. All data from one net tow in spring were excluded because MOCNESS nets did not open and close at the correct depths.

Counts of larvae from each taxon in each sample were converted to densities per 1000 m^3 and square-root-transformed to reduce the effects of rare high counts (Sokal and Rohlf 1995). For statistical modeling, densities were then normalized such that the sum of all values for each taxon and cruise was equal to one. The resulting unitless "relative larval density" metric expressed the contribution of each sample to the total density in that particular time series. The use of relative density values allowed for meaningful comparisons of vertical distributions among seasons with different absolute densities.

Linear models and GAMLSS models (generalized additive model of location scale and shape) were fit to the data, with relative larval density as the dependent variable and environmental factors at depth (including interaction terms) as explanatory variables. GAMLSS fits specific families of statistical distributions to empirical data by simultaneously optimizing parameters for the location (e.g., mean), scale (e.g., variance), and shape (e.g., skewness) of the distributions (Stasinopoulos and Rigby 2007; Hernandez et al. 2009). Ecological density data sets have two properties that make fitting most types of distributions difficult: (*i*) negative

Fig. 3. Examples of (*a*) spring, (*b*) summer, and (*c*) fall depth profiles of environmental variables measured in the Straits of Florida. Depth profiles were collected every 3 h for 42–48 h, and the presented profiles are not intended to be representative of the entire period. Gray shading corresponds to nominal depth ranges at which discrete ichthyoplankton samples were collected. Points indicate measurements taken at the midpoints of depth ranges, which were used to characterize samples in statistical models. Conversion factor: 1 μE = 1 μmol of photons.

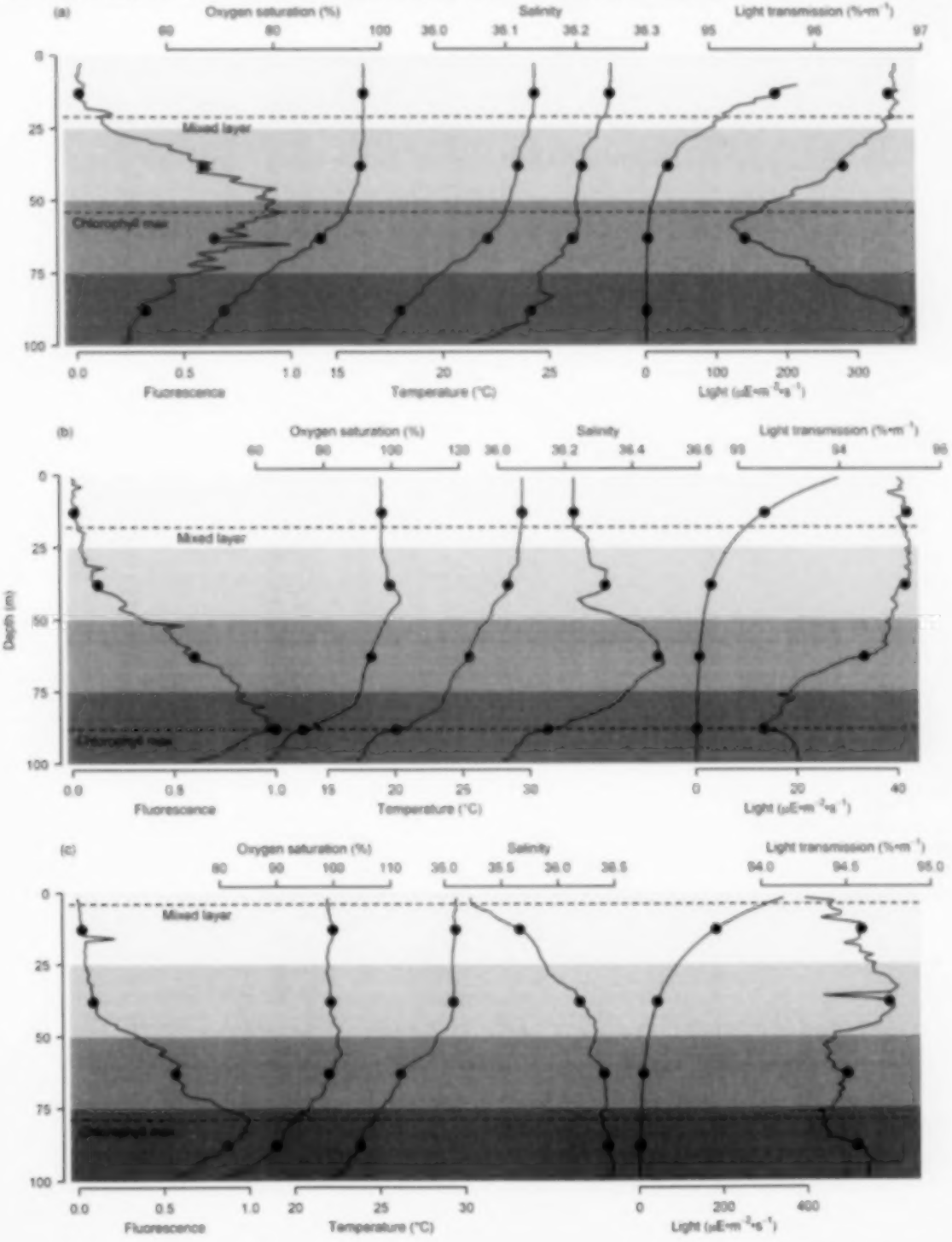


Table 3. Range of environmental variables characterizing spring, summer, and fall ichthyoplankton samples collected every 3 h in the Straits of Florida over 42–48 h from 0 to 100 m depth and used to predict (*i*) relative larval fish densities and (*ii*) larval fish depth.

			Spring		Summer		Fall	
Factor	Unit	Source	Min.	Max.	Min.	Max.	Min.	Max.
(*i*) Relative larval fish densities								
Chlorophyll fluorescence	(Arbitrary units)	CTD	0.09	1.33	0.05	0.64	0.09	0.80
Chlorophyll (relative)	%	CTD	6	54	6	59	6	55
Light-at-surface	$\mu E \cdot m^{-2} \cdot s^{-1}$	Ship	<15	1035	<15	995	<15	550
Light-at-depth[a]	$\mu E \cdot m^{-2} \cdot s^{-1}$	Ship, CTD	<0.2	635	<0.1	474	<0.2	277
Oxygen saturation	%	CTD	66	98	60	99	80	100
Depth	m	MOCNESS	10	91	11	92	10	98
Salinity	(Unitless)	MOCNESS	35.9	36.3	35.6	36.6	35.3	36.4
Temperature	°C	MOCNESS	16.6	25.5	15.4	29.6	21.3	29.4
Light transmission	$\%\cdot m^{-1}$	CTD	94	97	93	95	94	95
Zooplankton settled volume	$mL \cdot 1000\ m^{-3}$	MOCNESS	290	1140	190	800	260	1380
Zooplankton (relative)	%	MOCNESS	12	37	10	40	10	40
(*ii*) Larval fish depth								
Deep chlorophyll maximum	m	CTD	52	77	59	93	63	93
Light-at-surface	$\mu E \cdot m^{-2} \cdot s^{-1}$	Ship	<15	1035	<15	995	<15	550
Mixed layer depth	m	CTD	4	24	0	20	0	45
Oxygen saturation at 87.5 m[b]	%	CTD	66	90	60	96	80	96
Salinity at 12.5 m[b]	(Unitless)	MOCNESS	36.2	36.3	36	36.2	35.3	35.9
Temperature at 12.5 m[b]	°C	MOCNESS	24.5	25.5	29.3	29.6	29.0	29.4
Tidal phase index	(Unitless)	NOAA	−1.2	1.3	−1.2	1.2	−1.2	1.2
Wind speed	$km \cdot h^{-1}$	NOAA	1.5	21.7	2.2	15.6	2.6	17.7
Zooplankton center of mass	m	MOCNESS	44	56	43	54	39	53

Note: Factors included in models predicting relative larval fish density (*i*) represent individual samples, each measured at the mean depth of the sample (nominally 12.5, 37.5, 62.5, and 87.5 m). Factors included in models predicting larval fish depth (*ii*) characterize the state of the entire water column. CTD, conductivity–temperature–depth; MOCNESS, Multiple Opening Closing Net with Environmental Sampling System. Conversion factors: 1 μE = 1 μmol of photons.

[a]Light-at-depth was calculated from light-at-surface and light transmission at depth.

[b]Oxygen, salinity, and temperature measurements from depths with the greatest variability.

densities are meaningless, and (*ii*) densities of zero are common. To model these properties, we used zero-truncated *t* distributions and zero-inflated beta distributions in GAMLSS models. Nevertheless, the resulting GAMLSS models were generally driven by linear terms, and therefore equivalent to the much simpler linear models. Consequently, only the results of linear models are presented. Akaike's information criterion (AIC) was used as a guideline for selecting explanatory variables and interaction terms to include in the models. AIC expresses the degree to which a model captures the information contained in a data set while penalizing the model for each additional factor, thus limiting overparameterization. Since AIC does not measure the statistical significance of a model, the predictive value of models was tested by cross-validation. Samples from one season at a time were designated as "validation data," while samples from the remaining one or two seasons were used as "training data" for fitting a model. Each model was used to predict relative larval densities in the independent validation data, based on environmental variables only. Model significance was determined by a permutation test of the correlation between predicted and observed values (Hesterberg et al. 2005).

A second analysis was conducted with the depth at which larvae were collected as the response variable. This was accomplished by bootstrapping (drawing with replacement) samples of 10 000 larvae for each taxon from the original time series. The probability of drawing a larva from any particular sample was set to the square-root-transformed relative larval density, which is analogous to a square-root transformation of larval counts. The resulting bootstrapped samples were analyzed as described above by cross-validation of linear and GAMLSS models followed by permutation tests for significance testing. The strength of the second analysis was that variables characterizing the entire water column, as opposed to a specific sample, could be used as explanatory factors. Mixed layer depth, for example, could not have been used to predict larval densities in specific samples, but might nevertheless affect larval vertical distributions. The strength of the first approach was that the presence or absence of larvae from samples could be examined. In the second analysis this was impossible, because there was no way to calculate the depth of "absent fish."

Finally, to identify potential effects of environmental variability on ichthyoplankton assemblages, similarities in the taxonomic composition of samples were analyzed. Bray–

Table 4. Summary of linear models with significant (permutation test: $p < 0.05$) cross-validated value for predicting relative larval density from environmental factors.

Taxon	Season(s) used to fit model	Predicted season	Factor	R^2
Epinephelini	Spring and summer	Fall	Depth	0.50
	Spring and fall	Summer	Depth	0.39
	Summer and fall	Spring	Depth	0.15
Holocentridae	Spring and summer	Fall	Depth	0.31
	Spring and fall	Summer	Depth	0.44
	Summer and fall	Spring	Depth	0.36
Priacanthidae	Spring and summer	Fall	Depth	0.38
	Spring and fall	Summer	Depth	0.48
	Summer and fall	Spring	Depth	0.33
Pristipomoides spp.	Summer	Fall	Depth	0.40
	Fall	Summer	Depth	0.43
Sphyraenidae	Spring and summer	Fall	Depth	0.38
	Spring and fall	Summer	Depth	0.43
	Summer and fall	Spring	Depth	0.33
Apogonidae	Spring	Fall	Zooplankton	0.15
	Fall	Spring	Zooplankton	0.18
Sparisoma spp.	Spring and summer	Fall	Light-at-surface	0.10
	Spring and fall	Summer	Light-at-surface	0.23
	Summer and fall	Spring	Light-at-surface	0.10

Curtis dissimilarity matrices of square-root-transformed larval densities were used to perform Kruskal's NMDS (nonmetric multidimensional scaling). The Bray–Curtis index is widely used to measure ecological similarity across species and habitats, and NMDS is an ordination technique for visualizing the clustering of data in multidimensional space. A strength of NMDS is that unlike most ordination methods, NMDS neither requires that data meet restrictive parametric assumptions nor forces the clustering to conform to externally imposed explanatory variables (Legendre and Legendre 1998). This makes NMDS a powerful tool for exploratory data analysis of ichthyoplankton assemblages (e.g., Gray 1998). All data analyses were performed using the software package R (The R Project for Statistical Computing, http://www.r-project.org/).

Results

Larval composition

Over the course of three cruises, 8529 larvae representing at least 34 families strongly associated with coral reefs (Leis 1991*a*) were collected in MOCNESS samples (Table 1). Only larvae from the families Mullidae (n = 1279) and Tetraodontidae (n = 315) were collected in large numbers in neuston net samples, revealing that they aggregated at the surface of the water column. These two families were thus excluded from further analysis. Larvae from the most abundant groups could be identified to taxonomic levels ranging from 14 species identifications to one suborder. Most of these taxa are commonly observed on coral reefs in the Florida Keys (Bohnsack et al. 1999) and elsewhere in the Western Central Atlantic (Böhlke and Chaplin 1968). Various species in the subfamily Anthiinae, including the most abundant taxon of the study (*Hemanthias vivanus*), are common on reefs deeper than 70 m (Hastings 1981), but are less well known. Most species of the family Scorpaenidae (the second most abundant taxon) are associated with reefs, but some inhabit other benthic habitats.

Samples collected in summer and fall were more similar to each other, in terms of taxonomic composition of reef fish larvae, than to samples collected in spring (Fig. 1). At the same time, samples formed clusters following an easily recognizable gradient in depth range from the deepest (75–100 m) to the most shallow (0–25 m) (Fig. 1). The combined density (untransformed) of reef fish larvae per 1000 m^3 of water in 1 mm mesh MOCNESS samples from all depth ranges was 58 in spring, 19 in summer, and 46 in fall. In several taxa, densities were much higher during either spring or fall than in the other two seasons. For example, *Hemanthias vivanus* made up 48% of the combined density in spring but only 3% in summer and 2% fall. Scorpaenids made up <1% of the combined density in spring, 1% in summer, and 31% in fall. Combined densities of larvae excluding the two above taxa were quite similar among seasons: 30, 18, and 31 larvae per 1000 m^3 in spring, summer, and fall, respectively.

Larvae from 13 taxa fulfilled the sample size requirements for further data analysis. In each time series, larvae from each analyzed taxon were present in >62% of MOCNESS tows. Approximately half of these larvae were collected during the day and half during the night (Table 2). However, the density of *Sparisoma* spp. larvae in the daytime samples accounted for <20% of the total, indicating diel net avoidance in this taxon. Among the 13 taxa analyzed, larval densities were frequently highest at 0–25 or 25–50 m and relatively low at 75–100 m depth (Fig. 2). The only group with highest densities at 75–100 m depth were scorpaenid larvae sampled during the fall time series.

Fig. 4. Observed and predicted relative densities of coral reef fish larvae (square-root-transformed and normalized such that the sum of all values equals one) at different sampling depths. In spring, summer, and fall, the upper 100 m of the water column in the Straits of Florida was sampled every 3 h for two diel cycles. Predicted densities for each season were generated by linear models fit to observed data in the other seasons. Titles indicate the taxon and season being predicted. Observed values (○), lines, and associated R^2 values illustrate significant regressions of larval densities and sampling depth (permutation test: $p < 0.05$). Predicted values (×) and associated R^2 values indicate the significant accuracy of models (permutation test: $p < 0.05$). For each taxon, the seasons with highest and lowest model accuracy are shown.

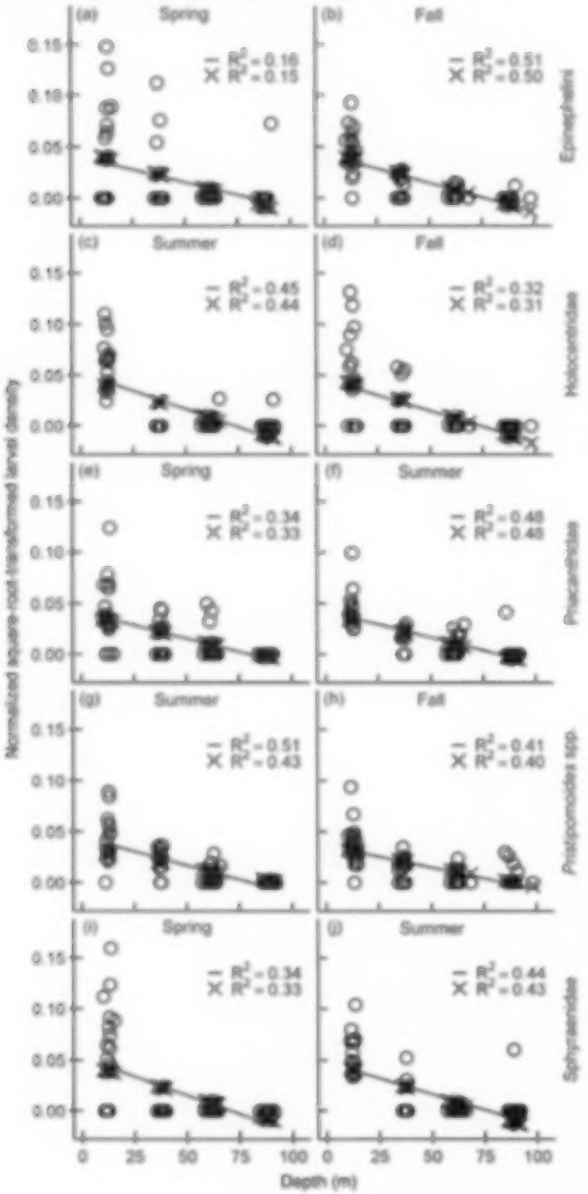
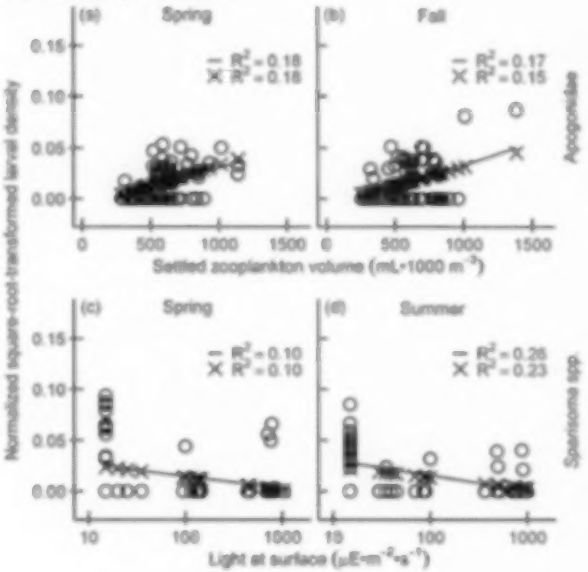


Fig. 5. Observed and predicted relative densities of reef fish larvae (square-root-transformed and normalized such that the sum of all values equals one), collected in three seasonal 42–48 h time series in the Straits of Florida at 0–25, 25–50, 50–75, and 75–100 m depth. Predictions were generated by linear models fit to data excluding the season being predicted. Titles indicate the taxon and season being predicted. The x axis specifies the explanatory environmental variable. Observed values (○), lines, and associated R^2 values illustrate significant regressions in the observed data (permutation test: $p < 0.05$). Predicted values (×) and associated R^2 values indicate significantly accurate predictions (permutation test: $p < 0.05$). For each taxon, the seasons with highest and lowest model accuracy are shown.

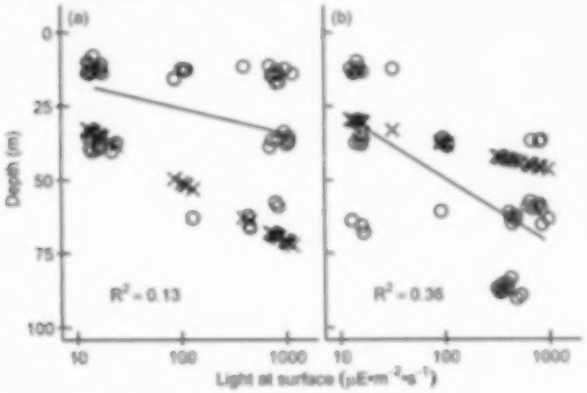


Statistical models

The environmental conditions under which larvae were collected varied substantially both within and among cruises (Fig. 3, Table 3). Most models generated using AIC (one model was fit per taxon per season that could be used as validation data) included multiple factors and interaction terms that only described the training data, but did not improve predictions for the validation data. The best cross-validated predictions were generally achieved by models using only one explanatory factor.

Models with significant (permutation test: $p < 0.05$) and cross-validated (at least two sampling periods) predictions for relative larval density were achieved in seven out of the 13 analyzed taxa (Table 4). Correlations were primarily driven by the presence or absence of larvae in different samples and to a lesser extent by differences among positive densities. Depth was the single most predictive factor for Epinephelini, Holocentridae, Priacanthidae, *Pristipomoides* spp., and Sphyraenidae, with all but one outlier having $R^2 \sim 0.31$–0.50 (Fig. 4). In some time series, relative densities of the above taxa were also related to other factors, such as oxygen saturation and light, but in all cases, the other factors were strongly correlated with depth (Supplemental Tables S1, S2[3]) and had less predictive value than depth.

Fig. 6. Random subset of bootstrapped observed (○) and predicted (×) depths of pomacentrid (excluding the genera *Abudefduf* and *Chromis*) larvae sampled in the Straits of Florida in spring (n = 44), summer (n = 47), and fall (n = 75) at 0–25, 25–50, 50–75, and 75–100 m depth. Regressions (lines) of surface light and observed depth were significant in each season (permutation test: $p < 0.05$), the strongest and weakest relationships are shown: (*a*) spring, (*b*) summer. Data from each combination of two seasons were used to predict larval depth as a function of surface light in the third season. Models correctly predicted the direction of regression lines, but were not accurate in predicting observed values. Observed data were jittered to reveal individual data points (small random numbers were added to each coordinate).



Predictable correlations between relative larval densities and settled zooplankton volume (but not zooplankton normalized to the entire water column) were present for apogonids (Fig. 5; spring: R^2 = 0.18; summer: no data; fall: R^2 = 0.15). Relative densities of larval *Sparisoma* spp. were negatively correlated with surface light (Fig. 5; spring: R^2 = 0.10; summer: R^2 = 0.23; fall: R^2 = 0.10), but not with light-at-depth, further indicating substantial diel net avoidance.

Cross-validated models accurately predicting larval depth were not achieved for any taxa. In the unique case of pomacentrids, the depth of larvae was significantly positively correlated with surface light in all three seasons (spring: R^2 = 0.13; summer: R^2 = 0.36; fall: R^2 = 0.18; permutation test: $p < 0.05$), revealing a diel vertical migration in this group (Fig. 6). Pomacentrid larvae consistently occupied shallower depth ranges during the night than during the day. However, both diel and nocturnal distributions were somewhat different in each season, causing model predictions to be inaccurate.

Discussion

Vertical distributions are vital to the ecology of marine fish larvae because the biological and physical factors that define their environment change dramatically with depth. Since the early days of vertically stratified ichthyoplankton sampling (e.g., Ahlstrom 1959), it has been clear that larval vertical distributions can be dynamic yet somewhat predictable. Unfortunately, very few studies (e.g., Heath et al. 1988; Hernandez et al. 2009) have attempted to quantitatively predict distributions. All taxa in the present study have physoclistic swimbladders, which means that larvae can actively maintain neutral buoyancy at depth (e.g., Govoni and Hoss 2001), beginning at an early developmental stage (around yolk-sac absorption and first feeding) (Pelster 2004), while directly controlling their vertical movements by swimming (e.g., Leis 2004; Huebert and Sponaugle 2009). The degree to which different environmental variables are predictive of larval vertical distributions thus provides an empirical estimate for their importance in stimulating vertical swimming responses.

Depth-regulating behavior

Of the environmental factors examined here, depth was the single best predictor of densities of reef fish larvae, resulting in significant cross-validated predictions for five taxa. Including other explanatory variables in addition to depth did not improve the precision of model predictions. All five depth – larval density relationships were quite stable in the face of substantial environmental variability, including seasonal changes in temperature, diel changes in light, and stochastic changes in turbulence. Among the taxa Epinephelini, holocentrids, priacanthids, *Pristipomoides* spp., and sphyraenids, depth models predicted a mean 35% of total variability in larval densities, including variability caused by horizontal patchiness among different parcels of water passing by the sampling station. It is possible to remove horizontal variability and focus entirely on vertical patterns by comparing predicted and observed proportions of larvae among each set of four net samples comprising one MOCNESS tow. Depth models of this kind predicted a mean 51% of vertical variability in larval proportions for the same five taxa.

Given accumulations of larvae at consistent depths in otherwise variable environments, which cues may be driving larval vertical orientation and swimming behavior? Our findings provide circumstantial evidence that larvae orient via hydrostatic pressure. While we treated depth and pressure as synonymous in our sampling and analyses, depth (distance from the surface) and pressure (force per area exerted by surrounding water) can be distinguished given proper ecological context. Pelagic fish larvae are frequently found at depths from which they cannot perceive the ocean surface, either by mechanoreception, vision (particularly at night), or any other known sense. However, larvae can sense hydrostatic pressure (Qasim et al. 1963; Govoni and Hoss 2001), and depth-regulating behavior of coral reef fish larvae in response to pressure cues has been demonstrated in controlled laboratory experiments conducted inside hyperbaric chambers (Huebert 2008). Larval behavioral preferences for specific levels of hydrostatic pressure thus present a parsimonious explanation for predictably stable larval vertical distributions.

All taxa with significant depth – larval density relationships accumulated at 0–25 m, with few larvae caught >50 m depth. This common pattern (Heath 1992) is often interpreted as an effect of the pycnocline (e.g., Palomera 1991; Coombs et al. 2001), sometimes without rigor-

[1] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

ous hypothesis testing. While there is strong experimental evidence that some larvae avoid low temperature (Olla et al. 1996) or high salinity (Lougee et al. 2002), many larvae aggregate in the upper 50 m independent of the strength, location, or even presence of a pycnocline (Conway et al. 1997; Olivar and Sabates 1997). In our study, the main pycnocline started at a depth of ~70 m in spring, ~30 m in summer, and ~50 m in fall and continued past the 100 m limit of MOCNESS sampling in all seasons. No consistent effects of either temperature or salinity on larval distributions were apparent.

Factors related to feeding and predation

Other environmental factors that are frequently used to explain larval vertical distributions in the sea include light, turbulence, and zooplankton concentration, all of which affect larval feeding. Obviously, high concentrations of zooplankton prey are favorable for feeding. As visual predators, larvae also depend on light to detect, pursue, and attack their prey (Blaxter 1986; Job and Bellwood 2000). Small-scale turbulence increases the rate of random prey encounters (Rothschild and Osborn 1988), but can interfere with successful prey capture when strong (MacKenzie and Kiorboe 2000). Given at least some light and some prey, vertical gradients in the above factors must cause a particular depth range to be most favorable for feeding. For example, in temperate continental shelf waters with strong seasonal stratification, the favorable range can correspond to the mixed layer (Buckley and Lough 1987), indicating once again the potential role of the pycnocline. In oceanic water around Barbados, the favorable range for *Thalassoma bifasciatum* is correlated with the deep chlorophyll maximum layer (Cowen et al. 2003). Similar logic can be applied to predation on larvae; thus, a particular depth range may also be most favorable for avoiding predators, and larvae may ultimately distribute in such a way as to optimize feeding and predation (Fortier and Harris 1989; Pearre 2003). Unfortunately, it has proven particularly difficult to obtain reliable measurements of predation (reviewed in Bailey and Houde 1989). Assuming that our measurements captured feeding and predation conditions, reef fish larvae did not generally adjust their vertical distributions in response to changing conditions within the three sampling periods.

The depth at which invertebrate zooplankton was most highly concentrated was not predictably related to the depth at which fish larvae were concentrated for any taxon. However, tows in which the concentration of zooplankton was unusually high across multiple depths were significantly and positively correlated with high relative densities of sampled apogonid larvae. In other words, while apogonid larvae did not accumulate at depths rich in zooplankton, they did accumulate in the entire water column under high zooplankton conditions. The observed relationship is therefore indicative of a horizontal as opposed to vertical pattern. Since our location was fixed for the duration of each sampling period, we sampled different water masses as they passed by the sampling station. Florida Current frontal eddies are known to accumulate both fish and invertebrate zooplankton off the Florida Keys (Limouzy-Paris et al. 1997; Lane et al. 2003; Richardson et al. 2009), and correlated fluctuations in zooplankton and apogonids may have been caused by passing water masses previously associated with eddies. This hypothesis does not explain why relative densities of other taxa were not predictably affected. Correlations between zooplankton and relative larval density were present in spring *Hemanthias vivanus* as well as spring and summer pomacentrids, but not in summer or fall *H. vivanus* or fall pomacentrids (thus not cross-validated).

Our only finding consistent with potential predator–prey-related vertical movements was a significant correlation between surface light and the depth at which we collected pomacentrid larvae in all three seasons. However, models did not accurately predict larval depths because of differences in vertical distributions among seasons. In other words, models predicted the presence and sign of a significant linear regression, but not the slope or intercept of the regression line. The pattern did not suggest behavioral preferences for specific light levels because relative pomacentrid densities were unrelated to light-at-depth. Instead, a transition between the daytime distribution and the nighttime distribution coincided with changes in light. Zooplankton (including fish larvae) commonly use dusk and dawn as cues for synchronizing vertical migrations thought to enhance feeding during the day (at the risk of increased predation) and reduce predation at night (Forward 1989; Neilson and Perry 1990; Richards et al. 1996). In our case study, pomacentrid densities increased at 50–75 m depth during the day and increased at 0–25 m depth during the night. Since pomacentrids require daylight to feed (Job and Bellwood 2000), the nocturnal distribution may be related to predator avoidance but not feeding. The diurnal distribution may be related to predator avoidance or prey densities or both.

Net avoidance

The daytime net avoidance by *Sparisoma* spp., evident in the small percentage of larvae collected during the day, remains unexplained. There are two ways that larvae could have avoided capture during the day. Either *Sparisoma* spp. moved to depths that were not sampled by the MOCNESS, or they escaped the approaching sampling gear. *Sparisoma* spp. larvae were exceedingly rare in neuston net samples and thus did not avoid MOCNESS nets by accumulating at the ocean surface. Larval densities at 75–100 m depth were consistently low, including samples collected around dawn and dusk, suggesting that larvae did not migrate to deeper depths during the day. However, migrations to depths >100 m cannot be entirely ruled out since these depths were not sampled. The remaining explanation, visual net evasion, is common for some sampling methods (Ahlstrom 1959; Heath 1992), but surprising for a net with 4 m^2 mouth opening towed at ~1.5 $m \cdot s^{-1}$. While many settlement-stage reef fish larvae can swim at speeds of 0.4 $m \cdot s^{-1}$ for short periods of time (reviewed in Leis 2006), swimming performance is related to the propulsive area of the fins (Fisher et al. 2000); thus, small larvae are much slower swimmers. *Sparisoma* spp. larvae were not larger or more developed than other larvae in our samples, and it seems unlikely that they consistently escaped nets when no other taxa did.

Unexplained variability and limitations of the study

One of our most striking results was the amount of variability in larval distributions that was unrelated to a broad

range of environmental factors. Larvae almost certainly have sufficient swimming abilities (reviewed in Leis 2006) to assume vertical distributions matching their environmental preferences. However, for about half of the analyzed taxa, we found no compelling evidence that larvae sought out specific environmental conditions within the range encountered in the Straits of Florida. There are several possible explanations for this finding. First, it is possible that some exogenous factors did not repeatedly vary over sufficiently large ranges within our data set, limiting our ability to effectively cross-validate models. The presence of greater environmental extremes in more than one time series may have resulted in more predictable behavioral responses of larvae. Second, the 25 m vertical resolution of our samples may have been insufficient to detect some types of potentially important patterns. For example, the vertical distributions of phytoplankton (e.g., Rines et al. 2010) and invertebrate zooplankton (e.g., Young et al. 2009) can be patchy on much finer spatial scales (~1 m), and the effects of such thin layers of plankton on fish larvae would not have been detectable by our methods. Third, unpredictable vertical distributions may reflect behavioral preferences for environmental factors we did not address. For example, the vertical distributions of specific prey species were not resolved by our methods, and predators were not addressed at all. Fourth, larvae may exhibit seasonal variation in behavioral preferences. A multiyear data set would be required for cross-validations in this case. Finally, unpredictable vertical distributions may reflect behavioral plasticity within taxa, with different subsets of larvae exhibiting unique environmental preferences. Our study was limited to the detection of consistent environmental effects, but endogenous biological factors characterizing individual larvae may have caused the same environmental conditions to be favorable to some larvae and unfavorable to others.

Endogenous factors likely to interact with exogenous environmental variables in determining vertical orienting and swimming behavior include species, developmental stage, and satiation. With the exception of *H. vivanus*, the species composition within taxa analyzed in this study may have varied among seasons, and the presence of multiple species with different behaviors may have hindered the effectiveness of cross-validations. The lack of predictable patterns for pooled groups of multiple species is therefore inconclusive, and stronger patterns may emerge from species-level identifications, possibly by genetic techniques (e.g., Richardson et al. 2007). On the other hand, the presence of predictable patterns in some groups of larvae pooled at higher taxonomic levels indicates that closely related species often do behave similarly. Larval size and developmental stage also may be important. For example, postflexion larvae tend to occupy deeper depths than preflexion larvae around Barbados (Cowen 2002). Finally, even among individuals of the same species and size, variations in satiation may lead to different vertical behavior (Pearre 2003). With respect to our data, this aspect could perhaps be addressed by larval gut content analysis (e.g., Llopiz and Cowen 2009).

Regional implications

Since the geographic ranges of reef fishes in the Western Central Atlantic generally span the entire Caribbean region and beyond, it may be possible to extrapolate from our study in the Straits of Florida to other locations in the region. While it would be imprudent to expect vertical distributions elsewhere to exactly mirror those in the Straits of Florida, larval behaviors are likely to be equivalent, since gene flow among Caribbean reef fishes limits their potential for local adaptations (Planes 2002). Two patterns evident in our data set are likely to be of general regional importance. First, strong behavioral preferences for specific levels of hydrostatic pressure may be common among reef fish larvae. Specifically, preferences for relatively low levels of pressure in several taxa, including representatives of the commercially important grouper (Epinephelini) and snapper (*Pristipomoides* spp.), should result in predictable accumulations of larvae at <25 m depth. Second, within the range of environmental conditions addressed in our study, strong behavioral preferences for exogenous factors other than pressure (i.e., light, oxygen, phytoplankton, salinity, temperature, tidal phase, turbulence, water clarity, and zooplankton biomass) may generally be uncommon. However, this could change when the same larvae are exposed to more extreme conditions, both in the Straits of Florida and elsewhere in the Western Central Atlantic. For example, turbid freshwater intrusions associated with North Brazil current rings affect the vertical distributions of reef fish larvae in oceanic waters off Barbados (Cowen et al. 2003). With respect to more extreme environments, our measurements could be used to generate hypotheses for the range of conditions that fall within behavioral preferences as well as environmental tolerances of reef fish larvae.

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Potential for domesticated–wild interbreeding to induce maladaptive phenology across multiple populations of wild Atlantic salmon (*Salmo salar*)

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Abstract: We report how aquaculture may negatively alter a critical phenological trait (developmental rate) linked to survival in wild fish populations. At the southern limit of the species range in eastern North America, the persistence of small Atlantic salmon (*Salmo salar*) populations may be constrained by interbreeding with farmed salmon that escape regularly from intensive aquaculture facilities. Using a common-garden experimental protocol implemented over an 8-year period, we show that embryos of farmed salmon and multigenerational farmed–wild hybrids (F_1, F_2, wild backcrosses) had slower developmental rates than those of two regional wild populations. In certain cases, our data suggest that hybrid developmental rates are sufficiently mismatched to prevailing environmental conditions that they would have reduced survival in the wild. This implies that repeated farmed–wild interbreeding could adversely affect wild populations. Our results therefore reaffirm previous recommendations that based on the precautionary principle, improved strategies are needed to prevent, or to substantially minimize, escapes of aquaculture fishes into wild environments.

Résumé : Nous démontrons comment l'aquaculture peut affecter négativement un trait phénologique essentiel (le taux de développement) lié à la survie des populations sauvages de poissons. À la limite sud de l'aire de répartition de l'espèce dans l'est de l'Amérique du Nord, la persistance de petites populations de saumons atlantiques (*Salmo salar*) peut être restreinte par des croisements avec des saumons d'élevage qui s'échappent régulièrement des installations d'aquaculture intensive. À l'aide d'un protocole expérimental de jardin commun maintenu sur une période de 8 années, nous montrons que les embryons de saumons d'élevage et des hybrides de plusieurs générations de saumons sauvages et élevés (rétrocroisements en nature de F_1 et de F_2) ont des taux de développement plus lents que les poissons de deux populations régionales sauvages. Dans certains cas, nos données indiquent que les taux de développement des hybrides sont suffisamment mésappariés aux conditions prévalentes de l'environnement qu'ils auraient réduit la survie en nature. Ceci donne à entendre que des croisements répétés entre des poissons d'élevage et des poissons sauvages pourraient affecter négativement les populations sauvages. Nos résultats nous amènent donc à réaffirmer les recommandations antérieures basées sur le principe de précaution voulant que des stratégies améliorées soient nécessaires pour empêcher, ou réduire à un strict minimum, la fuite de poissons d'élevage vers les environnements sauvages.

[Traduit par la Rédaction]

Introduction

Unparalleled declines of many wild fish populations, coupled with growing aquaculture production, have made it critical to assess the potential risks associated with interactions between wild and escaped farmed fishes (Naylor et al. 2005). From a genetic perspective, a primary concern is the potential loss or reduction of adaptive genetic variation that may arise in wild fishes if farmed–wild interbreeding occurs. Indeed, the domestication process can elicit genetic changes in farmed fishes that are maladaptive in the wild, and recent studies support that farmed–wild hybrids may have reduced survival (McGinnity et al. 2003; Tymchuk et al. 2007; Fraser et al. 2008) or genetically based phenotypes that likely reduce their fitness performance relative to wild fish (Einum and Fleming 1997; Fraser et al. 2010).

A more rare aspect of phenotype considered within the literature of farmed–wild interactions has been phenology. Many wild species of fishes, especially migratory ones, need to coordinate their movements and shifts in life history stages with the timing of seasonal resource availability in geographically separated areas (Crozier et al. 2008; Helfman et al. 2009). For instance, fish embryonic developmental

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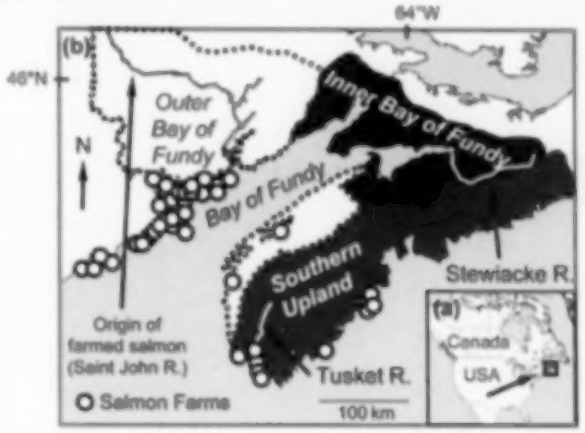
rates (i.e., the cumulative number of degree-days prior to hatch) often have a partial genetic basis and appear to be finely tuned to local environmental conditions that are optimal for growth and survival (Beacham and Murray 1987; Jensen et al. 1991; Donaghy and Verspoor 1997). As a result, if escaped farmed fishes differ from their wild counterparts in such phenological traits, farmed–wild hybrids might not synchronize their development with the timing of critical seasonal events.

We address this possibility with a consideration of embryonic developmental rates in Atlantic salmon (*Salmo salar*) populations at the southern limit of their range in the Northwest Atlantic, many of which are already endangered or declining in the USA and Canada because of a variety of human activities (US Atlantic Salmon Assessment Committee 2005; Committee of the Status of Endangered Wildlife in Canada 2006). This region also harbours intensive Atlantic salmon aquaculture. Large escapes of farmed salmon from sea cages are recurrent, with escapees entering many rivers inhabited by wild salmon, sometimes at a high rate (≥10%–20% of returning adults; Morris et al. 2008). Regional escapees can spawn successfully in the wild (Carr et al. 1997) and interbreed with wild salmon (O'Reilly et al. 2006).

Embryonic developmental rates might differ genetically between farmed and wild salmon in the Northwest Atlantic for at least two, nonmutually exclusive reasons. First, farmed salmon originate chiefly from the Saint John River, a watershed of higher altitude and latitude than many regional rivers where escaped farmed salmon have been found (Morris et al. 2008) (Fig. 1). Owing to colder water and (or) longer winters, higher altitude–latitude populations often spawn earlier in autumn and have slower embryonic developmental rates than lower altitude–latitude populations (Beacham and Murray 1987, 1990; Webb and McLay 1996), although the developmental rate of the wild Saint John progenitors that generated the farmed strain is unknown. Second, artificial selection on regional farmed salmon may either intentionally or inadvertently result in genetically based changes to developmental rates from the wild state. For instance, in an analogous situation, data from cultured hatchery fish have shown that slower, genetically based developmental rates can be favoured (Reisenbichler and Rubin 1999). Additionally, available data on adult broodstock selection in fish farms and hatcheries also shows that it tends to favour those adults that spawn earlier to fill yearly production quotas (McLean et al. 2005; Bert 2007). Such earlier spawning individuals often produce offspring with slower developmental rates (Beacham and Murray 1987).

We adopted a common-garden experimental protocol to test whether, in southeastern Canada, differences existed in embryonic developmental rates among farmed salmon, wild salmon from two populations, and their multigenerational farmed–wild hybrids (i.e., F_1 = farmed × wild; F_2 = F_1 × F_1; wild backcrosses = F_1 × wild). Separated by ≈340 km, the wild populations, from the Tusket and Stewiacke rivers, were each representative of a regional group of related populations known as "Southern Upland" and "Inner Bay of Fundy", respectively (Fig. 1). These regional groups exhibit several differences in migratory behavior, life history, and genetic characteristics (Committee of the Status of Endangered Wildlife in Canada 2006; Fraser et al. 2007, 2010). They have also experienced dramatic population declines since the 1990s, with the Inner Bay of Fundy now assigned an "endangered" conservation status (Fisheries and Oceans Canada 2002; Committee of the Status of Endangered Wildlife in Canada 2006). Escaped farmed salmon have been detected historically in both the Tusket (≈1%, 1998) and Stewiacke (≈33%, 1995) rivers, although their presence in these and other regional rivers is likely underestimated (see Morris et al. 2008). Owing to the small size and critical conservation status of regional wild populations, it was not feasible to carry out our research in the wild.

Fig. 1. Maps of (*a*) the general study location within eastern North America and (*b*) the location of Atlantic salmon study populations, regional groups of wild populations, and the general location of regional salmon farms.



We examined two generations of interbreeding because adverse genetic changes arising from intraspecific hybridization might not be manifested until the F_2 generation when parental genes are recombined (Edmands 2007). While our study was not designed to disentangle the influence of artificial selection on developmental rates during farming from that of the farmed ancestry, our farmed salmon originated from the major strain used in regional salmon aquaculture (Saint John River). Hence, from a regional risk-assessment perspective, our study is representative both of the farmed salmon that escape into the wild and the probable farmed–wild breeding interactions that occur.

Materials and methods

Study populations and crosses

In 2001, unfertilized eggs and sperm were collected from sexually mature adults originating from the Tusket River, the Stewiacke River, and the Saint John River farmed strain, which at that point had undergone four generations of artificial selection primarily for faster growth and delayed maturity (Glebe 1998) (Fig. 1). Adults represented random samples from each population or broodstock. Tusket adults were obtained from the wild; Stewiacke adults had been collected as juveniles in the wild and subsequently raised to sexual maturity in captivity. Gametes were transferred to

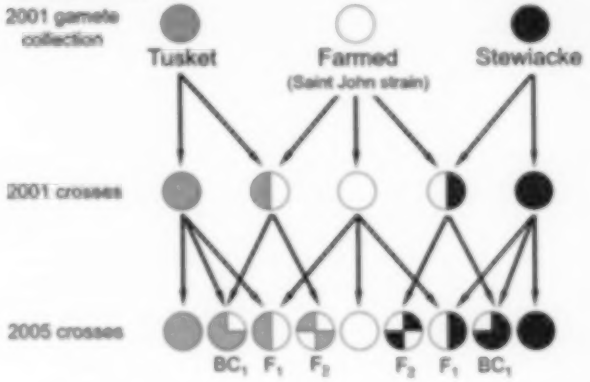
Dalhousie University's Aquatron Facility and used to generate 10 full-sibling families each of the three parental and two F_1 farmed–wild hybrid crosses (i.e., F_1 farmed–Stewiacke hybrids, with parentally reciprocal families in equal numbers, and F_1 farmed–Tusket hybrids with farmed females and Tusket males only; Lawlor et al. 2009; Fig. 2). Crosses generated in 2001 were raised under common environmental conditions until they attained maturity in 2005. Details of 2001–2005 rearing conditions, as well as genotyping and parentage analyses of 2005 adults back to their respective 2001 families (to avoid inbred matings in 2005 crosses) are described in Fraser et al. (2010). The 2005 adults were used to regenerate the pure and two F_1 farmed–wild hybrid crosses, as well as to create two F_2 farmed–wild hybrids and two farmed–wild backcrosses (BC_1) (Fig. 2). Crosses were performed on 22, 25, and 29 November and 6 December 2005. Each cross consisted of 9–23 chiefly full-sibling families (see Supplemental Appendix S1[1]), except for Tusket and both F_2 hybrids in which each male was crossed to two or three different females, because of logistical constraints in generating large numbers of families in these crosses with available sexually mature fish. Details on the sex of wild, farmed, or hybrid parents used to generate the multigenerational crosses (F_1, F_2, BC_1) in 2005 are also found in Supplemental Appendix S1[1].

Rearing

The 2005 families comprised ≈500 eggs and were randomly allocated to one of three compartments nested within one of 60 circular 100 L tanks that received the same flow-through water source. Thus, our study made the assumption that any tank effects were equal across the crosses generated; ongoing work further suggests that tank effects at this facility have a negligible impact on developmental rates (D.J. Fraser and J.A. Hutchings, unpublished data). Compartments were of equal size, separated by equal distances, and had their bottoms and sides open and covered with a thin-mesh screen to ensure sufficient oxygenation for the eggs and hatching alevins. Under common environmental conditions (e.g., temperature ±0.1–0.15 °C, pH = 7.0), eggs were kept in the dark until hatching commenced in March 2006, and dead eggs were removed every 4 days during incubation. Temperatures were recorded daily for each tank throughout the incubation period to determine cumulative degree-days prior to hatching.

We cannot discern whether our rearing conditions might be more similar to one parental environment or the other (see McClelland and Naish 2007). Nevertheless, importantly for the study of developmental rates, our incubation temperatures approximated those to which regional wild salmon are naturally exposed annually (2–4 °C; Lacroix 1985; Houde 2009). Namely, the mean water temperature over the entire incubation period was 4.5 °C (±0.12 °C, standard error, SE) and water temperature was allowed to fluctuate naturally over the incubation period (between 3 and 6 °C). Water originated from a natural watershed near Dalhousie University (Pockwock Lake, at a latitude between Tusket and Stewiacke rivers). Potential rearing environment influences on developmental rates should therefore have been minimized.

Fig. 2. General structure of the experimental cross design and crosses generated.



Nonetheless, experimental pH (7.0) more closely resembled conditions to which farmed and Stewiacke salmon were normally exposed (pH = 6.0–6.5; Tusket pH = 4.6–5.2) (Fraser et al. 2008).

Embryonic developmental rates

We recorded the number of individuals that hatched in each family every 24 h throughout the incubation period. Given the large number of eggs per family ($n \approx 500$), digital photos were taken daily to accurately count newly hatched alevins. Owing to slight daily temperature differences between tanks (range: 0.10–0.15 °C), it was necessary to standardize the numbers of individuals hatching at any given degree-day across families. To do so, the minimum number of degree-days accumulated within 24 h over all families was used as a binning interval (4 degree-days). Then, the number of individuals hatching in each family was calculated for each 4-degree-day interval; thus, the mean cumulative degree-days to hatching for each cross could be determined for the analyses below.

Statistical analyses

Mixed effects analysis

We analyzed developmental rates based on individual counts of eggs that hatched in every 4-degree-day interval. The purpose was to draw inference on whether differences in developmental rates existed among farmed, wild, and hybrid crosses. Data were nonindependent at the family level, so we chose a hierarchical mixed effects analytical framework that accounted for the within group (family) correlation when estimating differences at the cross level (Diggle et al. 2003). Letting Y_{ijk} be the cumulative degree-day to hatch for egg i in family j, cross type k, a linear mixed effects model describing the response at both the cross and family level was written as

$$Y_{ijk} = \mu_0 + \Delta\mu_k + b_j + \varepsilon_{ijk}$$

where μ_0 was the overall fixed effect mean degree-day to hatch (set to be the mean response of one of the wild populations, see below), $\Delta\mu_k$ was the fixed-effect deviation from

[1] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

μ_0 by cross k, b_j was the random effect deviation from $\mu_0 + \Delta\mu_k$ by family j with b_j assumed to be normally distributed $b_j \sim N(0, \sigma_b^2)$, and ε_{ijk} was the deviation of the individual egg from the average for family j, $\varepsilon_{ijk} \sim N(0, \sigma_\varepsilon^2)$. The significance of the estimated $\Delta\mu_k$ away from zero indicated whether there was a cross-difference from μ_0, while b_j provided for the nonindependence of the response at the family level. Models were run for each of the farmed–wild crosses, i.e., one for farmed–Stewiacke crosses and one for farmed–Tusket crosses (Supplemental Appendix S2[3]). As an example, the model run for the farmed–Tusket crosses (equivalent for the farmed–Stewiacke comparison) was

$$Y_{ijk} = \mu_{\text{TUSK-mhd}} + (\Delta\mu_k + b_j) + \varepsilon_{ijk}$$

where k = {F_1 hybrid, F_2 hybrid, BC hybrid} from the farmed–Tusket crosses, and $b_j = \{b_1, b_1, \ldots, b_{58}\}$ were the random effects per family (i.e., the total number of Tusket, farmed, and farmed–Tusket hybrid families).

Variance decomposition

We assessed whether variance in developmental rates differed among farmed, wild, and hybrid crosses. The variance was decomposed in three ways. First, separate variances of the random effects per cross would indicate whether crosses differed in the spread of the familial response. This scenario was modeled by letting $\sigma_b^2 = \sigma_{bk}^2$ so that a separate random effects variance was estimated per cross k. Second, the residual variance could differ among crosses. This was modeled by decomposing the residual variance by $\sigma_\varepsilon^2 = \sigma_{\varepsilon k}^2$. To check whether particular families influenced the differences of the residual variance between crosses, we thirdly placed a random effect on the residual variance so that a residual variance per cross, as well as the effect of family, could be accounted for. This was modeled using

$$\sigma_\varepsilon^2 = \sigma_{\varepsilon k}^2 \exp(z_j)$$

where $z_j \sim N(0, \sigma_z^2)$. Note that the use of the exponential prevents negative estimates of the variance, while a family within a cross could have a variance that is any proportion of the overall cross residual variance.

Inclusion of potential maternal effects

In salmonid fishes, early-life history traits can be influenced by maternal effects (e.g., Beacham and Murray 1990; Einum and Fleming 2000). Thus, when crosses differed from one another in embryonic developmental rates within a farmed–wild comparison, we carried out supplementary analyses to account for potential maternal effects (included as fixed effects). This was assessed using the subsets of families in different crosses that originated from the same mothers (a full description of these analyses is found online in Supplemental Appendix S3[3]). Note that owing to our cross design (Supplemental Appendix S1[3]), maternal effects could not be accounted for between some crosses.

Transformations

Preliminary data plotting revealed that some families exhibited negative skew, with longer tails to the left of the hatch distribution (Supplemental Appendix S1[3]). Biologically, we interpreted this as a nontrivial contribution of early hatchers in some families to the overall distribution of cumulative degree-days to hatch. Though not severe (the probability density was largely symmetric), we investigated the effect of the skew on the overall results by data transformation (details online in Supplemental Appendix S4[3]).

Maximum likelihood was used to fit the models, which were then compared using Akaike's information criterion (Akaike 1973). The models were implemented in both AD-Model Builder RE Module (ADMB-RE; Otter Research Ltd. 2005) and SAS PROC NLMIXED (SAS Institute Inc. 2004). Maximum likelihood can produce downward bias in the standard errors of the estimates (used for the line cross analysis below), so the models were also fit via restricted maximum likelihood for comparison. Standard errors of the estimates differed negligibly between the restricted and unrestricted maximum likelihood fits. Our large sample size ensured that the maximum likelihood values tend to be unbiased in this case.

Line cross analysis

As a final step to our analyses, the joint scaling based on least squares regression (Lynch and Walsh 1998) was used to evaluate the fit of our cumulative degree-day data in each farmed–wild comparison (i.e., cross means and variances) to a simple additive model of genetic differentiation between parental populations. No further model building involving nonadditive effects (e.g., an additive-dominance model) was conducted because the simple additive model could not be rejected (both $\chi^2 \leq 1.54$, df = 3, $P \geq 0.67$). Note that the joint-scaling test assumed trait normality, which was not entirely met with our data. It was also based on a diploid model of inheritance but Atlantic salmon exhibit residual tetraploidy. Nevertheless, a suitable genetic model that can account for such a mode of inheritance currently does not exist (Fraser et al. 2008).

Results

Embryos of farmed salmon had slower developmental rates (cumulative degree-days to hatch) than embryos from each wild population (Tusket, Stewiacke) (all $P < 0.0001$; Table 1; Fig. 3; Supplemental Appendix S2[3]). The magnitude of this difference was greater between farmed and Tusket salmon than between farmed and Stewiacke salmon (Table 1; Fig. 3).

Hybrids (F_1, F_2, BC) in both farmed–wild comparisons exhibited developmental rates that were intermediate between parents and hence slower than in wild populations (Table 1; Fig. 3). A simple additive genetic model adequately explained the differences between parental and hybrid crosses (farmed–Stewiacke: $\chi^2 = 0.71$, df = 3, $P = 0.87$; farmed–Tusket: $\chi^2 = 1.54$, df = 3, $P = 0.67$). In particular, both F_1 farmed–wild hybrids differed in developmental rates from their respective wild parents, as did one second generation hybrid (farmed–Tusket backcrosses) (Table 1; Fig. 3). Removing mothers used more than once in different families within crosses did not affect the results (Supplemental Appendix S2[3]). Furthermore, cross differences in embryonic developmental rates remained where potential maternal effects could be accounted for (i.e., when the same mothers were used in multiple crosses) (Supplemental Appendix S3[3]). Familial contributions to the overall distribution of

Table 1. Results of the linear mixed effects analysis (with family as a random effect) to test for differences in embryonic developmental rates between crosses.

Cross	Avg. cumulative degree-days to hatch	SE	*t*	df	*p*
Farmed–Stewiacke					
Wild	527.62	3.39	—	—	—
Farmed	549.29	2.94	4.71	75	<0.0001
F_1 hybrid	535.62	2.68	1.89	75	0.0631
F_2 hybrid	528.43	2.19	0.21	75	0.8379
BC hybrid	527.54	2.37	–0.02	75	0.9849
Farmed–Tusket					
Wild	504.91	2.84	—	—	—
Farmed	549.29	2.94	11.58	56	<0.0001
F_1 hybrid	528.24	3.12	5.86	56	<0.0001
F_2 hybrid	509.68	2.87	1.27	56	0.2102
BC hybrid	511.93	3.02	1.82	56	0.0747

Note: The *t* statistic reflects differences from the wild population. The degrees of freedom (df) are the number of families minus two (two random effects in model — see Supplemental Appendix S2, available online at http://cjfas.nrc.ca). SE, standard error; BC, backcross.

hatching times of each cross are presented in Supplemental Appendix S1[3].

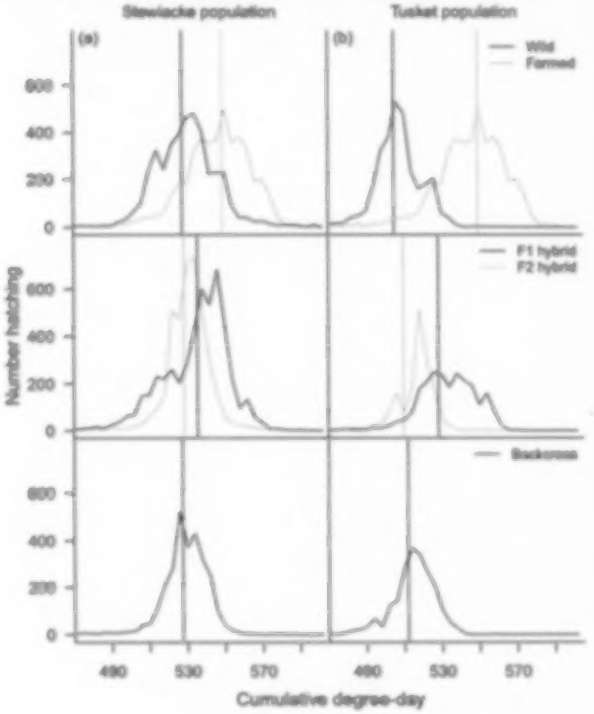
Finally, we found little evidence that variance in developmental rates differed between crosses. Significant differences were observed in the residual variances by cross, but once a random effect on the variance by family was included in the analysis, the differences became nonsignificant. This indicated that particular families within crosses drove the apparent differences in variance of the residuals. Incorporating separate, random effects variances on the mean hatch date in the analysis also did not result in any detectable differences among any of the crosses (Supplemental Appendices S1, S2[3]).

Discussion

Our demonstration of likely heritable differences in embryonic development rates among Atlantic salmon populations was not unexpected based on previous studies of salmonid fishes (e.g., Beacham and Murray 1987; Jensen et al. 1991; Donaghy and Verspoor 1997). Our demonstration of genetically based phenotypic differentiation between multigenerational farmed–wild hybrids and their wild salmonid counterparts is also consistent with previous studies (e.g., McGinnity et al. 2003; Tymchuk et al. 2007; Fraser et al. 2010). What distinguishes our study is the documentation of phenological trait differences in a consistent direction among farmed salmon, two wild salmon populations, and their multigenerational hybrids, within a region where farmed salmon escape repeatedly into the wild while wild salmon are severely depleted. Specifically, in the Northwest Atlantic, the farmed Atlantic salmon strain most commonly used in aquaculture had slower developmental rates than two at-risk wild Atlantic salmon populations representative of regional wild fish. In both farmed–wild comparisons studied, hybrids most likely to be produced in the wild (F_1) had slower developmental rates than wild salmon (with $P <$ 0.05 in the farmed–Tusket comparison and $P = 0.06$ in the farmed–Stewiacke comparison). Second generation hybrids most likely to be produced in the wild (i.e., wild backcrosses; Edmands 2007) also exhibited this same trend ($P =$ 0.07) in the farmed–Tusket comparison. The observed differences in mean developmental rates between regional farmed–wild hybrids and wild salmon (8–22 degree-days for F_1 hybrids) or between regional farmed and wild salmon (22–45 degree-days) are within or slightly greater than the range of values reported in wild-only studies of salmonids conducted at comparable spatial scales (e.g., Beacham and Murray 1987, 1990; Berg and Moen 1999).

The conservation status of regional wild salmon populations precluded experimentation in the wild. We are thus inherently restricted to making inferences about the consequences of the observed developmental rate differences beyond the laboratory. Nevertheless, the magnitude of the observed differences in mean developmental rates between regional farmed–wild hybrids and wild salmon in our study may be of biological significance in the natural environment. On average, F_1 hybrids would be expected to hatch 2–11 days later than wild salmon in nature, based on mean

Fig. 3. The distribution of developmental rates (cumulative degree-days to hatch) for farmed–wild hybrids relative to parental crosses. (*a*) Stewiacke vs. farmed, (*b*) Tusket vs. farmed. Fitted averages of the lowest Akaike information criterion (AIC) mixed effects model, which takes family into account, are presented as vertical lines with the same line type as the distribution. Egg numbers per cross were as follows: Stewiacke (4164), Tusket (3140), farmed (4443). Farmed–Stewiacke hybrids: F_1 (5140), F_2 (8241)*, backcross (5735)*. Farmed–Tusket hybrids: F_1 (2358), F_2 (1734), backcross (2117). *Egg numbers halved for the purpose of plotting.



winter incubation temperatures of 2–4 °C within regional rivers (Lacroix 1985; Houde 2009), which closely resemble the mean temperature under which our experimental research was carried out (4.5 °C). In streams, salmon hatching precedes emergence from the gravel, the point at which young alevins have exhausted the energy stores within maternally provided yolk sacs (Beacham and Murray 1987, 1990). We were unable to precisely quantify emergence times, but available data on other salmon species suggest that later-hatching salmon also emerge later (Beacham and Murray 1987, 1990). Later emergence can negatively impact alevin growth rate (Metcalfe and Thorpe 1992), early survival (Einum and Fleming 2000), territory acquisition (Harwood et al. 2003), and later life-history events linked to survival (e.g., timing of smolt migration; Garcia de Leaniz et al. 2007). Importantly, such consequences may arise in nature even when emergence is delayed by only a few days (e.g., Einum and Fleming 2000). Given that regional farmed escapes are recurrent (Morris et al. 2008), and even low levels of farmed–wild gene flow can shift wild phenotypes away from the wild optimum (Ford 2002), repeated farmed–wild interbreeding could ultimately result in a reduction in the average fitness of wild individuals and a concomitant reduction in abundance of regional wild populations (Fleming et al. 2000; McGinnity et al. 2003).

Regional river temperatures can increase to 8–10 °C between the time of hatching and emergence (Lacroix 1985; Houde 2009), and so the magnitude of the difference between farmed–wild hybrids and wild salmon (in terms of numbers of days) might be offset partially by narrowing the gap in hatch time between them. Furthermore, other early life history traits linked to embryonic development rates might help to offset adverse effects of delayed hatching in farmed–wild hybrids if, relative to wild fish, such hybrids hatch or emerge at a larger size, grow faster, or exhibit more aggressive-dominant behaviours. Available data suggests that this might only occur in one of two wild populations studied. Tusket salmon were larger (at yolk absorption), grew faster (up to 400 days after hatching), and were more aggressive than F_1 and backcross farmed–Tusket hybrids (Fraser et al. 2010; Houde et al. 2010*a*). Regardless, farmed–wild hybrids of both wild populations exhibit a maladaptive expression of other traits important to fitness, including reduced antipredator responses (Houde et al. 2010*b*) and reduced acid tolerance (see Fraser et al. 2008).

We also have no evidence that F_1 farmed–wild hybrid alevins might still hatch at the same and presumably optimal time as wild alevins in spring because farmed females spawn earlier than wild females, or because farmed–wild hybrids inherit from their farmed parents a capacity to develop faster than wild salmon in warmer winter years. First, under common environmental conditions, males and females from all three of our parental populations (farmed, Tusket, Stewiacke) overlapped in the dates at which they became ready to breed. Second, Darwish and Hutchings (2009) compared developmental rates between Tusket and farmed–Tusket backcrosses at three temperatures higher than those studied here (5.3, 9.2, and 15.4 °C) and found little evidence that backcrosses develop faster with increasing temperatures.

Finally, one of two F_1 hybrid crosses in our study (farmed–Tusket) did not include parentally reciprocal families, as wild females and farmed males were not crossed together. Such families might not exhibit as slow a developmental rate as F_1 hybrids composed of farmed females and wild males; a visual inspection of reciprocal familial contributions to the overall distribution of hatching times in F_1 farmed–Stewiacke hybrids raises this possibility (Supplemental Appendix S1[3]). Yet mating between wild males and farmed females, as was most represented in our two F_1 hybrid crosses, may be more representative of what takes place in the wild (Fleming et al. 2000).

Our results underscore the necessity for future modeling to evaluate the demographic consequences of farmed–wild interbreeding for regional wild populations, under scenarios of both recurrent and episodic intrusions of farmed escapes (Hindar et al. 2006). For instance, developmental rates of hybrids most likely to be produced in the wild (F_1, wild backcrosses; Edmands 2007) were intermediate between their respective parents. Thus, overall, altered developmental rates from farmed–wild interbreeding would likely revert back to the wild state if a one-time high rate of interbreeding (e.g., through a single, large escape event) or only very low levels of interbreeding occur. Nevertheless, before a later generation fitness rebound could occur (i.e., F_1 and beyond), a drop in hybrid fitness in the F_1 generation would be expected, which might itself reduce the probability of persistence of wild populations currently at an elevated risk of extinction.

In conclusion, we have illustrated how aquaculture, a human activity undergoing rapid, worldwide expansion, may genetically alter a critical phenological event related to survival across different wild populations — in this case, populations of a migratory species, which may be particularly sensitive to phenological mismatches (Stenseth and Mysterud 2002; Crozier et al. 2008). Given the growing number of species being domesticated for human purposes (Ellstrand 2003; Hutchings and Fraser 2008; Kidd et al. 2009), our study reinforces previous recommendations for management strategies to prevent domesticated–wild interbreeding (Ellstrand 2003; Thorstad et al. 2008 and references therein) as an essential step toward retaining the appropriate genetic composition needed for phenological matching in the wild.

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The effect of flow regime on the occurrence of interference and exploitative competition in a salmonid species, white-spotted char (*Salvelinus leucomaenis*)

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Abstract: We examined the effect of flow regime on the occurrence of interference and exploitative competition in a salmonid species, white-spotted char (*Salvelinus leucomaenis*). In the lotic treatment, char showed typical consequences of interference competition (i.e., fish aggressively defended their foraging positions, and individuals occupying the most profitable positions grew fastest). In the lentic treatment, however, char behavior was consistent with exploitative competition; fish cruised over a larger area in search of food resources and showed limited aggression and no evidence of a dominance hierarchy. Our results indicate that white-spotted char showed interference competition more commonly than exploitative competition in the lotic habitat and vice versa in the lentic habitat.

Résumé : Nous examinons l'effet du régime d'écoulement sur la présence de compétition d'interférence et d'exploitation chez une espèce de salmonidé, l'omble à taches blanches (*Salvelinus leucomaenis*). Dans les conditions expérimentales d'eau courante, les ombles affichent les conséquences typiques de la compétition d'interférence (c'est-à-dire que les poissons défendent avec agressivité leurs sites d'alimentation, et les individus occupant les sites les plus favorables croissent le plus vite). En revanche, dans des conditions expérimentales d'eau stagnante, les poissons explorent de grandes surfaces en quête de ressources alimentaires, manifestent peu d'agressivité et il n'y a pas d'indication de hiérarchie de dominance. Nos résultats indiquent que l'omble à taches blanches présente plus communément une compétition d'interférence qu'une compétition d'exploitation dans l'habitat lotique et l'inverse dans l'habitat lénitique.

[Traduit par la Rédaction]

Introduction

Interference and exploitative competition are two of the primary modes of competition (Miller 1967). Interference competition is characterized by interindividual interactions (e.g., aggression) that occur when individuals compete for habitat resources. This mode of competition occurs commonly when nearby foraging territories differ in their quality. Interference competition can lead to differences in growth rate among individuals as dominant individuals monopolize food resources in profitable foraging territory. In contrast, exploitative competition is characterized by a lack of interindividual interaction when individuals scramble for food resources. Exploitative competition may not lead to notable differences in growth rate among individuals because of a lack of competitive dominancy.

Recent studies have highlighted the effect of density conditions on the occurrence of exploitative and interference competition in salmonid species (e.g., Grant and Imre 2005; Lobón-Cerviá 2007). For example, using young-of-the-year individuals, Grant and Imre (2005) demonstrated that interference competition is less common than exploitative competition at low densities because habitat resources are not limiting. However, the effects of habitat and food resource characteristics on the occurrence of either mode of competition are poorly understood. This is surprising as these resources are closely related with the occurrence of competition.

Most salmonid species inhabit both lotic and lentic habitats (e.g., Biro et al. 1997; Beechie et al. 2005). The foraging and social behavior of salmonid species differs between these habitats because of differences in the pattern of food supply. In lotic habitats, individuals occupy a foraging territory and ambush drifting invertebrates. The occupying individual will generally defend the territory against entry by other individuals (Fausch 1984; Elliott 1990). Conversely,

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in lentic habitats, salmonid species are rarely territorial or aggressive. Instead, individuals cruise large distances for seeking food items because points of food input such as benthic or floating invertebrates are often unpredictable (Biro et al. 1997; Sotiropoulos et al. 2006).

We hypothesized that resource characteristics affect the occurrence of interference or exploitative competition via their effect on fish behavior. Although behavior may be closely related to the modes of competition, previous studies have not evaluated the relationship between them. In lotic habitats, the upstream side should be more desirable, as foraging efficiency is higher (i.e., increased likelihood of encountering drifting invertebrates) at the local habitat scale (Fausch 1984; Nakano 1995). Thus, the quality of foraging territories often differs markedly in the lotic habitats. As a result, such habitats are likely to favor the occurrence of interference competition. Fish should aggressively defend their foraging territories and individuals that occupy the upstream sites should grow faster. As a result, the difference of growth rate among individuals may occur notably because of food monopolization by dominant individuals. In contrast, territory defense is unlikely to have a large effect on foraging efficiency in lentic habitats. Instead, individuals should cruise over a large area to obtain adequate food resources. An increase in cruising distance is an indicator of the occurrence of exploitative competition because individuals would scramble for food resource through cruising behavior. Because competitive dominancy does not occur as a result of exploitative competition, there is often little difference in growth rate among individuals. To test these hypotheses, we conducted experiments in artificial streams using white-spotted char (*Salvelinus leucomaenis*) as a model species.

Materials and methods

Artificial streams and experimental fish

We used six artificial concrete streams that were located at the National Research Institute of Fisheries Science (NRIFS) in Nikko, Tochigi Prefecture, Japan. Each stream was 6 m long × 2 m wide × 1 m high. The water inlet and outlet (0.82 m width) was located at the center of upstream and downstream ends, respectively. We controlled the current velocity in each stream using a baffle that was attached to the inlet. Water depth was controlled using a wood panel at the outlet. We placed a mesh screen (mesh size: 14 mm × 7 mm) in front of the wood panel to prevent fish escaping. Each stream was covered with a 30 mm clear-mesh net to protect the fish from predators such as herons (*Ardea cinerea*). The artificial streams were supplied with water (9.4–11.4 °C) from Jigoku Stream, a small spring-fed stream running through NRIFS. The artificial streams were located on both sides of the drainage channel (three streams per side).

Experimental char were chosen from captive bred fish in NRIFS. They originated from the Naka River, Tochigi Prefecture, Japan, and have been reared in NRIFS since 1965 (Masaoka et al. 1997). Hatchery and wild salmonids typically display similar patterns of aggressive and feeding behavior, although the intensity and efficiency of these behaviors may differ between these two groups (Weber and Fausch 2003; Yamamoto et al. 2008). Thus, our results are likely applicable to wild char.

Table 1. Mean current velocities ($cm \cdot s^{-1}$) for each transect in each reach.

	Lotic treatment			Lentic treatment		
Reach	1.5 m	3 m	4.5 m	1.5 m	3 m	4.5 m
1	6.32	6.01	5.99	0.54	1.09	1.10
2	6.67	5.96	5.46	1.07	0.54	0.54
3	6.06	5.76	5.53	0.54	0.55	0.54
df	2	2	2	2	2	2
F	0.60	1.98	0.04	0.51	0.01	0.01
P	0.45	0.17	0.84	0.48	0.96	0.99

Note: Current velocity on each line was not significantly different within each treatment (one-way analysis of variance, ANOVA, $P > 0.05$).

Study design

The experiments were conducted 3–18 July 2008. The six artificial streams were divided randomly into two treatments, lentic and lotic (three reaches per treatment). For the purposes of analysis the streams are referred to as lentic 1, 2, and 3 or lotic 1, 2, and 3. In each reach, we measured the current velocity at 10 randomly selected points along three transects, located 1.5, 3, and 4.5 m from the upstream margin. There was no difference in current velocity within each treatment (Table 1). The water depth was maintained at 0.2 m in all reaches throughout the experiment. We placed 15 fish into each reach (1.25 fish·m^{-2}) to simulate natural rearing densities for fish of this size (Grant and Kramer 1990). The fish were acclimated to the artificial streams for 2 days prior to the experiments. Prior to stocking, the fish were anaesthetized using ethyl aminobenzoate, and each fish was marked using two ribbon tags, which were attached between the head and the dorsal fin. Individuals were identified by the unique color combination of the tags. In addition, we measured the length and weight of each fish. The mean body length (range: 168.6–171.2 mm) and body weight (range: 66.2–71.7 g) were not significantly different among the six reaches at the beginning of the experiment (one-way analysis of variance, ANOVA; body length: df = 4, $F = 0.53$, $P = 0.47$; body weight: df = 4, $F = 1.11$, $P = 0.29$). Two percent of the total initial fish body mass of pellets (approximately total 20 g; particle diameter 3 mm) was supplied to each reach every day. Pellets were equally supplied four times (i.e., 5 g per feeding occasion) at various times during the daytime (08:00–18:00). To simulate natural conditions, the pellets were released in a single pulse at the inlet of the lotic treatment reaches or spread throughout a randomly selected area (1 m × 1 m) in the lentic treatment reaches. The fish were fed by hand by a single person to minimize disturbance. We did not observe the fish clustering towards the pellets at the time of feeding. The number of terrestrial invertebrates that fell into the artificial streams was extremely small. Therefore, we assumed that external inputs had no effect on fish behavior and growth during the experiment. To identify the location of each fish during the behavioral observations, each reach was divided into a grid consisting of 48 squares (0.5 m × 0.5 m).

We measured the body weight of each individual at the end of the experiment. The growth rate of each fish was then estimated using a formula for the specific growth rate (SGR):

$$SGR = 100 \cdot (\ln BW_2 - \ln BW_1) \cdot t^{-1}$$

where BW_1 and BW_2 represent the body weight (g) at the start and end of the experiment, respectively, and t is expressed in days (16 days). We calculated the range of SGR (the difference between highest and lowest SGR) in each reach to evaluate the differences in growth rate among individuals (Hasegawa and Yamamoto 2009). We compared the differences in growth rate among individuals between lotic and lentic treatments to evaluate the occurrence of either mode of competition. After the experiment, the fish were transferred back to the aquaculture ponds.

Behavioral observations

The fish were fed (5 g) immediately prior to the each observation period. We documented the behavior of fish six times for each reach during the experiment. The observations for each reach were randomly made within at least 2-day intervals between each observation. During each observation period, we conducted focal animal observations (Altmann 1974) of 10 randomly selected fish. Each fish was observed for 2 min. We recorded foraging attempts (defined as any movement to intercept a pellet) and aggressive behavior that was initiated by the focal individual. We also recorded the location of the fish (based on the position of the snout) within the square every 20 s. We calculated an index of movement by counting the number of squares between two observations and summing these over the 2 min observation window. Movements to any of the adjacent eight squares were regarded as "one square movement". We excluded movements that were clearly not directed towards foraging, such as chasing other individuals or escaping from an aggressor.

Data analysis

The data for foraging attempts, aggression, and movement distance during each observation period were skewed and contained a number of "zero" values. Therefore, we used the 20th quintile to represent the behavior of fish during each observation period. We evaluated differences in behavior between the lotic and lentic treatments and between observation periods using a repeated measure two-way ANOVA. The relationships among individual SGR and initial body size (body weight), behavior factors (foraging attempts, aggression, movement distance), mean distance from the upstream margin (number of squares from the upstream margin), and the location of the fish during each observation period (only in the lotic treatments) were tested using Kendall's correlation test for each reach. For each individual, we calculated a mean value for each behavioral measure based using data from all observation periods. We excluded the data from individuals that were observed less than three times (≥13 individuals were used in the analysis for all reaches).

During the 11th–13th days of the experimental phase, one or two fish died in three reaches (one reach in the lotic and two reaches in the lentic treatments). The dead fish were replaced with fish of a similar size to maintain constant density. The replacement fish were not used for behavioral observations and SGR analysis. The alpha level was set at 0.05 for all analyses.

Table 2. Results of repeated measure two-way analysis of variance (ANOVA) comparing foraging attempts, aggression, and movement distance over observation period at different flow regimes (lotic or lentic).

Effect	df	F	P
Foraging attempts (ε = 0.514)			
Observation period	2.57	0.167	0.893
Observation period × flow regime	2.57	1.277	0.329
Flow regime	1	0.111	0.756
Aggression (ε = 0.296)			
Observation period	1.48	1.078	0.375
Observation period × flow regime	1.48	1.078	0.375
Flow regime	1	153.0	<0.001
Movement distance (ε = 0.511)			
Observation period	2.56	0.778	0.514
Observation period × flow regime	2.56	0.155	0.901
Flow regime	1	11.49	0.028

Note: Degrees of freedom (df) were compensated by Greenhouse–Geisser's epsilon (ε) for observation period and observation period × flow regime.

Results

Effects of flow regime on behavior associated with competition

The data sets for foraging attempts, aggression, and movement distance were not consistent with an assumption of sphericity. Therefore, we adjusted the degrees of freedom for observation period and an interaction between observation period and flow regime (lotic or lentic) using Greenhouse–Geisser's epsilon (ε) (Table 2). Our results suggest that neither the observation period nor the interaction between the observation period and the flow regime affected fish behavior (Fig. 1, Table 2). However, with the exception of the number of foraging attempts, fish behavior was apparently different between the lotic and lentic treatments (Fig. 1*a*, Table 2). We observed significantly more aggressive acts in the lotic treatment reaches than in the lentic treatment reaches (Fig. 1*b*, Table 2). Conversely, the fish cruised over a larger area in the lentic treatment reaches than in the lotic treatment reaches (Fig. 1*c*, Table 2).

Effects of flow regime on SGR

There was no clear trend in mean SGR within each reach. Furthermore, the range of SGR was similar between lotic and lentic treatments (Table 3). In the lotic treatment, initial body size, frequency of foraging attempts, frequency of aggressive behavior, and movement distance were not correlated with SGR (τ = 0.051–0.385, P = 0.106–0.847). The distance from the upstream margin of the reach tended to be negatively correlated with SGR (lotic 1: τ = –0.505, P = 0.008; lotic 2: τ = –0.510, P = 0.008; lotic 3: τ = –0.379, P = 0.062; Fig. 2), suggesting that individuals occupying upstream territories had higher SGRs. In the lentic treatment, there was no correlation between initial body size or any of the behavioral factors and SGR (τ = –0.007–0.359, P = 0.100–0.747).

Fig. 1. Twenty quintiles of (*a*) foraging attempts, (*b*) aggressive acts, and (*c*) movement distance for each observation period to each reach. Solid lines and solid symbols represent the lentic treatment, while open symbols and dotted lines represent the lentic treatment. Reach 1, circles; reach 2, triangles; reach 3, squares.

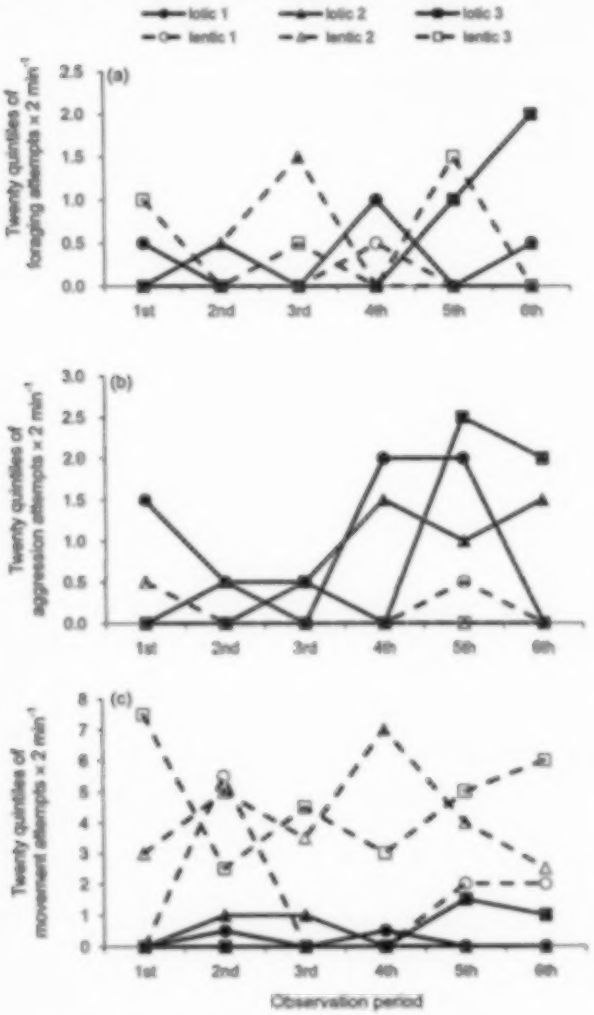


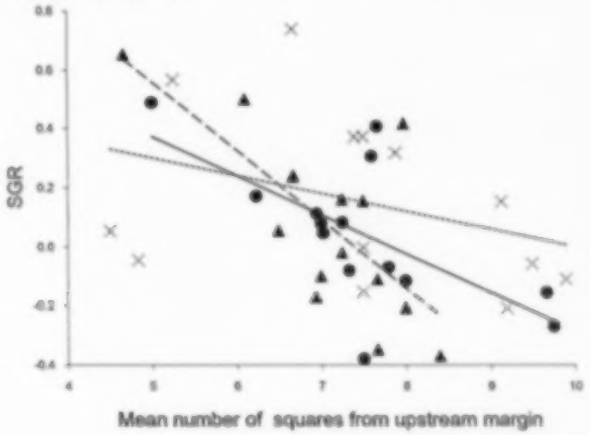
Table 3. Mean (± standard deviation, SD) and range of specific growth rate (SGR) in each reach.

	Mean (±SD)		Range	
Reach	Lotic	Lentic	Lotic	Lentic
1	0.068 (0.253)	0.060 (0.218)	0.868	1.100
2	0.074 (0.304)	0.225 (0.261)	1.024	0.811
3	0.156 (0.296)	0.188 (0.213)	0.949	0.880

Discussion

Our results clearly illustrate that the behavior of white-spotted char differed between the two habitat types (lentic and lotic). Aggressive behavior was more commonly observed in the lotic habitats than in the lentic habitats. Conversely, the char tended to cruise over a larger area in the lentic habitat than in the lotic habitat. It is generally accepted that this increase in cruising is a behavioral adaptation to improve foraging efficiency in stream-dwelling fish (e.g., Inoue et al. 2005). Cruising is indicative of the occurrence of exploitative competition, whereas aggressive behavior is an indicator of interference competition. Thus, our results suggest that lentic habitats favor exploitative competition, whereas lotic habitats favor interference competition.

Fig. 2. Relationship between individual specific growth rate (SGR) and the mean distance from the upstream margin (number of squares) for individuals during the observation periods (lotic treatment). Reach 1, circles and solid line; reach 2, triangles and dashed line; reach 3, × symbols and dotted line. $n = 14$ for reaches 1 and 2, $n = 13$ for reach 3.



The differences in the growth rate of experimental fish between the lotic and lentic treatments were not much different. Amount of food supply may not be limited enough to show the difference in growth rate. In particular, subordinate individuals in the lotic treatments might be able to consume sufficient food to grow. In the lotic treatments, however, we did note one of the typical outcomes of interference competition: that individuals who occupied the more profitable foraging territories (i.e., upstream) grew at a faster rate. As a result of intraspecific interference competition among salmonid species, large individuals generally become dominant and grow at a faster rate (Nakano 1995). However, we found no correlation between body size and growth rate, possibly because of compensatory growth in the smaller individuals (Elliott 1994). Alternatively, individual differences in the degree of acclimation to the experimental conditions may also affect the growth rate. For example, individuals that were able to acclimate more rapidly might be more likely to occupy profitable foraging territory, regardless of body size. The early establishment of territories is important, as prior residence is known to affect interference competitive dominancy in salmonid species (Huntigford and de Leaniz 1997).

In this experiment, we manipulated not only current velocity but also the spatial predictability of the food resource input. In natural lotic systems, these two variables (velocity and food) basically co-vary in the same way as in this experiment (e.g., Nakano 1995). However, the relation-

ship between these two variables differs in some natural lotic systems. For example, the pattern of flow is chaotic in structurally complex habitats where stones or woody debris are abundant. In these habitats, food may be delivered in a less predictable manner so that stream-dwelling salmonid species may not form obvious foraging territories. Thus, the results of our study may not be applicable to all lotic systems.

In the lentic habitat, the defense of foraging territory was not effective to sustain foraging efficiency, likely because the most desirable foraging habitat was not easily defined. Furthermore, we were unable to characterize dominant individuals based on growth rate in the lentic environments, a typical outcome of exploitative competition (Miller 1967). Salmonid species do not exhibit territoriality unless a given foraging position is defendable and qualitatively superior to others (Grant 1997). Instead, individuals maximize foraging efficiency by increasing cruising behavior. Individuals that are highly mobile may grow faster because of increases in their foraging rate (Grant and Noakes 1987). However, in some instances this is offset by energy loss due to cruising and (or) individual differences in the probability of encountering food items.

Competition regulates population density by changing mortality and emigration rates (Post et al. 1999; Fielding 2004; Moksnes 2004). In salmonid populations, previous studies have also suggested that density regulates population size by influencing the occurrence of interference and exploitative competition (Grant and Imre 2005; Lobón-Cerviá 2005, 2007). For example, Keeley (2001) demonstrated that fish densities decreased at high densities in artificial streams. This effect was caused by dominant individuals monopolizing the habitat and forcing the subordinate individuals to emigrate as a result of interference competition. In addition, our results suggest that the mechanisms responsible for population regulation may also differ depending on habitat and food resource characteristics.

Researchers have evaluated the effect of density on the occurrence of exploitative and interference competition using field data (e.g., Lobón-Cerviá 2007). However, the influence of habitat characteristics was, again, not considered in any of these studies. We conclude that interference competition is more common than exploitative competition in lotic habitat, and vice versa in lentic habitat. Our study is also unique in associating behavior with the occurrence of interference and exploitative competition. The influence of habitat characteristics and the supply of food resources should be taken into account when planning studies to evaluate competition (interference or exploitative). It is also likely that the results of previous studies can be re-interpreted, taking into account both density and resource (habitat and food) characteristics.

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Re-assessment of the stock–recruit and temperature–recruit relationships for Pacific sardine (*Sardinops sagax*)

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Abstract: The harvest guideline for Pacific sardine (*Sardinops sagax*) incorporates an environmental parameter based on averaged surface temperatures at the Scripps Institution of Oceanography pier (SIO pier) in La Jolla, California, USA, which would be invoked after a series of cool years to reduce commercial catches using a precautionary decision rule. We revisit the stock–recruit and temperature–recruit relationships underpinning the currently used environmental parameter for sardine assessment and found that the temperature–recruit relationship no longer holds for the SIO pier when time series are updated with data from more recent years. The significance of the correlation between temperature and recruitment was also artificially increased by autocorrelation in the time series. In contrast, the stock–recruit relationship was still valid when recent data were added. SIO pier surface temperatures are warmer than 10 m-depth Southern California Bight (SCB) temperatures where the sardine spawn, and the difference has increased since the late 1970s. Sardine recruitment was also not related to offshore temperatures in the SCB. We demonstrate that the environmental proxy derived from SIO pier temperature, which has never affected the harvest guideline since its implementation, no longer predicts recruitment of Pacific sardine, and should be removed from sardine management.

Résumé : Les directives pour la récolte des sardines du Pacifique (*Sardinops sagax*) incorporent une variable environnementale basée sur la moyenne des températures en surface au quai de la Scripps Insitution of Oceanography (SIO) à La Jolla, Californie, É.-U., qui pourrait être invoquée après une série d'années fraîches afin de réduire les récoltes commerciales en utilisant une règle décisionnelle préventive. Nous réexaminons les relations stock-recrues et température-recrues qui sous-tendent la variable environnementale couramment utilisée pour l'évaluation des sardines et trouvons que la relation température-recrues n'est plus valable pour le quai de la SIO quand les séries chronologiques sont mises à jour avec les données des années récentes. La signification de la corrélation entre la température et le recrutement est aussi artificiellement haussée par l'autocorrélation dans la série chronologique. En revanche, la relation stock-recrues reste valide après l'addition des données récentes. Les températures de surface au quai de la SIO sont supérieures aux températures à la profondeur de 10 m dans le golfe du Sud de la Californie (SCB) où les sardines se reproduisent et la différence a augmenté depuis la fin des années 1970. Le recrutement des sardines n'est pas non plus relié aux températures du large dans la SCB. Nous démontrons que la variable environnementale de remplacement dérivée de la température au quai de la SIO ne permet plus de prédire le recrutement des sardines du Pacifique et devrait être exclue de la gestion des sardines.

[Traduit par la Rédaction]

Introduction

The paleoceanographic record shows that Pacific sardine (*Sardinops sagax*) populations fluctuated greatly in biomass long before the advent of commercial fishing (Baumgartner et al. 1992). Sardine stocks are prone to collapse, contraction in range, and re-expansion, as well as to shifts in dominance in the small pelagic fish community (Schwartzlose et al. 1999). To an unknown degree, the changes in sardine abundance are driven by changes in their environment. There are very few fisheries stock assessments or harvest policies that incorporate any measure of environmental variability (Schirripa and Colbert 2006; Schirripa et al. 2009). The Pacific sardine harvest policy is unusual because a proxy for environmental variability, 3 year average surface temperature at the Scripps Institution of Oceanography pier

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(SIO pier) in La Jolla, California, USA, is used as one parameter in the formula for a decision-rule-based harvest guideline (Pacific Fishery Management Council 1998; Hill et al. 2008). There is considerable uncertainty as to the relative contributions of density-independent factors such as temperature, the distribution of water masses, and climatic processes operating at different scales (e.g., El Niño-Southern Oscillation cycles and "regime shifts", Lluch-Belda et al. (1989)) and density-dependent factors such as competition, predation and food supply. It is also unclear which stage of life is most affected by environmental variability. Spawning habitat is strongly impacted by environmental variability, but recruitment success is thought to be determined by natural mortality at the late larval or juvenile stage (Watanabe et al. 1995; Takahashi and Checkley 2008). Despite uncertainties about the mechanisms by which environment exerts an effect on sardine biomass and recruitment, the possible range of effects makes it desirable to incorporate an index of environmental variability into the Pacific sardine stock assessment.

The maximum sustainable yield (MSY) control rule is a proxy for F_{msy} (i.e., the fishing mortality rate that achieves equilibrium MSY) and is presently constrained to range between 5% and 15% of total biomass. The SIO pier temperature index values observed from 1981 through 2008 have consistently been larger than the temperature threshold that would trigger the decision rule to reduce the exploitation fraction and consequently the F_{msy} exploitation fraction has been 15%. This remains the case under current oceanic conditions. Essentially this means that the environmental parameter has no effect on the harvest guideline under current conditions, nor has it ever been used to reduce catches since its implementation in 2000, but if SIO pier temperature index values drop this could lead to a management decision to reduce the exploitation fraction.

The calculation of the exploitation fraction in the MSY control rule is very sensitive to temperature. A drop in the SIO pier temperature index of less than 0.5 °C from the 2009 value would shift the exploitation fraction from 15% to 5%. This would reduce the harvest guideline for Pacific sardine by 2/3 if other variables were constant. Since the consequences of such a reduction in harvest are substantial for the fishing industry, it is timely to re-examine the relationship between the SIO pier temperature index and the temperatures in the offshore Southern California Bight (offshore SCB) where sardine spawn, now that a further 17 years of data are available.

In this paper, we pose two questions. First, is the SIO pier temperature index still an appropriate proxy for environmental variability in the Pacific sardine assessment? To answer this, we examine temporal trends in surface temperatures at the SIO pier and compare them with trends in the offshore SCB where sardine spawn. Second, are relationships between Pacific sardine recruitment and SIO pier temperature that were based on data from 1935–1963 and 1985–1990 still valid? To address this question we examine the relationships between Pacific sardine recruitment anomalies, SIO pier temperatures, and offshore SCB temperatures incorporating updated data from 1991–2008.

Materials and methods

Our strategy was to test the temperature–recruit relationship for Pacific sardine, adjusting for stock size, using recruitment anomalies, rather than simply recruits, to emphasize deviations from any trend. First we tested the temperature–recruit relationship with data from the early years used by Jacobson and MacCall (1995), and then we re-tested after updating with 17 more recent years of data. Next, we examined the difference in trends between temperatures in the offshore Pacific sardine spawning area and the SIO pier. Finally, we retested the temperature–recruit relationship with the offshore temperatures to determine whether the temperature–recruit relationship worked with the temperatures from the sardine spawning grounds.

Environmental control rule for Pacific sardine

The formula for the harvest guideline is

$$HG_{yr+1} = (BIOMASS_{yr} - CUTOFF) \times FRACTION \times DISTRIBUTION$$

where HG_{yr+1} (in metric tonnes (mt)) is the total USA (California, Oregon, and Washington) harvest guideline in the year following the assessment, $BIOMASS_{yr}$ (mt) is the estimated July 1 stock biomass (ages 1+) from the current assessment, CUTOFF (150 000 mt) is the lowest level of estimated biomass at which harvest is allowed, FRACTION is an environment-based percentage of biomass above the CUTOFF that can be harvested by the fisheries, and DISTRIBUTION is the percentage of $BIOMASS_{yr}$, assumed to be 87%, in US waters. Given F_{msy} and the productivity of the sardine stock have been shown to increase when relatively warm-ocean conditions persist (Jacobson and MacCall 1995), the following formula has been used to determine an appropriate (sustainable) FRACTION value:

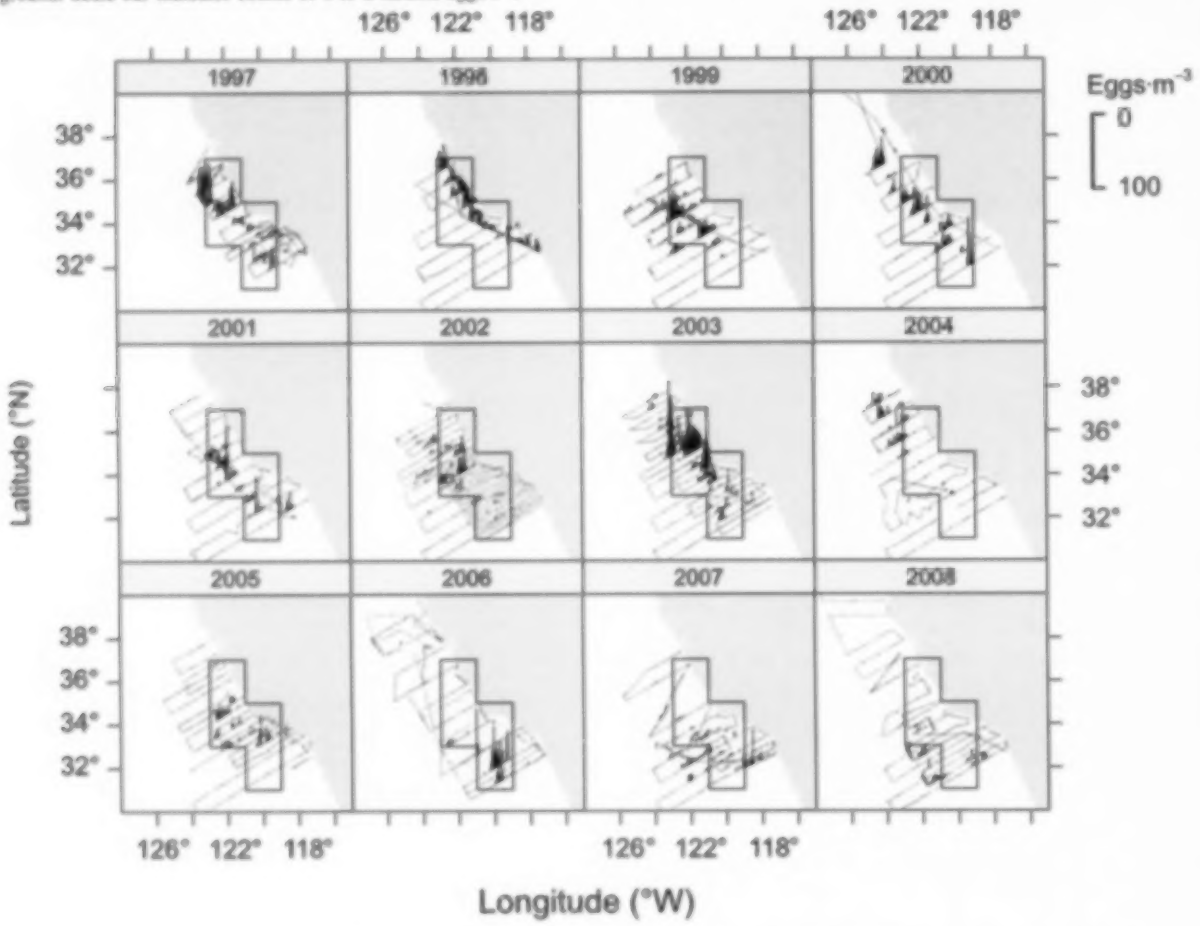
$$F_{msy} = 0.248649805(T^2) - 8.190043975(T) + 67.4558326$$

where T (°C) is the running average sea-surface temperature at the SIO pier during the three preceding seasons (July–June).

Comparison of SIO pier and offshore temperature trends

We assembled two temperature time series for analyses: one from the SIO pier and a second from the offshore SCB. The SIO pier temperature time series was obtained from the surface measurements of the Scripps Institution of Oceanography Shore Stations Program (shorestation.ucsd.edu). Daily temperature measurements were averaged by month for the time period 1916–2008 and a 3 year running average was calculated. The offshore SCB temperature data were obtained from the NOAA_ERSST_V3 data provided by the National Oceanic and Atmospheric Administration Office of Oceanic and Atmospheric Research Earth System Research Laboratory Physical Sciences Division, Boulder, Colorado, USA (Xue et al. 2003; Smith et al. 2008). These are monthly means of sea surface temperature for 2° × 2° grids from 1854 to the present. Data were extracted for four grid squares (Fig. 1) and averaged for each time point. The grid squares were chosen to overlap areas where larger numbers of sardine eggs are detected by Continuous Underway Fish Egg Sampling (Checkley et al. 1997) during the March–April spawning season. Although the distribution of sardine

Fig. 1. Locations of the 2° × 2° grid squares in the Southern California region from which the offshore Southern California Bight temperature time series was extracted (denoted by heavy black lines). The grid squares are overlaid on counts of sardine eggs·s^{-1} collected in March–April–May 1997 to 2008 using the Continuous Underway Fish Egg Sampling system to show the location of the sardine spawning ground. Scale bar indicates counts of 0 to 2 sardine eggs·s^{-1}.



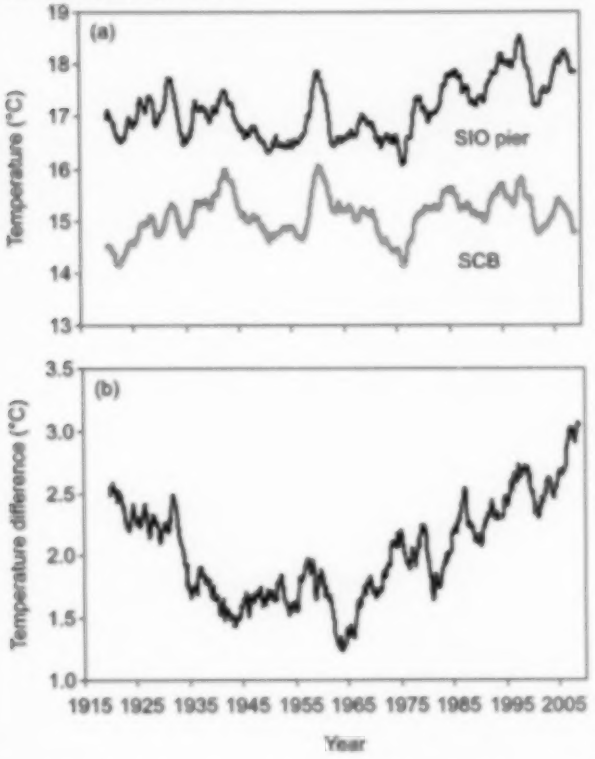
eggs shows large interannual variability, the spatial positions of higher egg counts (>2 eggs·min^{-1}) were well contained by the grid squares we used to determine temperatures in the offshore SCB sardine spawning area (Fig. 1). The exceptions to this generalization were in 2000 and 2004, when the eggs extended further north, and in 2007 and 2008 when there sardine eggs were found offshore as well as inshore of the grid square boundaries (Fig. 1).

SIO pier temperature and Pacific sardine recruitment

In the original work on the SIO pier temperature index (Jacobson and MacCall 1995), sardine recruitment (R) was related to spawning stock biomass (SSB) and SIO pier temperature (averaged over either 3 years or 5 years) using a generalized additive model (GAM) for the years 1935–1963 and 1985–1990. Their preferred management model used a LOWESS fit (Cleveland 1979) to the stock–recruit relationship ($\ln R$ on SSB) and a linear fit to $\ln R$ on the 3-year averaged surface temperature at the SIO pier (Jacobson and MacCall 1995). Jacobson and MacCall's (1995) stock–recruit relationship for Pacific sardine was based on Virtual Population Analysis estimates and age-2 recruits (1935 to 1963) or age 1 recruits (1985 to 1990) corrected to age-2 using estimated mortality. Numbers of age-2 fish were used to estimate recruits to permit comparison with data from the early years. We also used age-2 fish as recruits to permit comparison with Jacobson and MacCall (1995), although the current assessment (Hill et al. 2008) treats age-0 fish as recruits. We updated Jacobson and MacCall (1995) results with more recent data from the Stock Synthesis 2 (SS2) integrated assessment model, lagging R by two years in relation to SSB. We re-evaluated both the stock–recruit relationship and the temperature–recruit relationship (adjusting for stock size) after including more recent data collected between 1991 and 2008. We fitted the stock–recruit relationship for Pacific sardine with a Ricker curve using both the early data (1935–1963 and 1985–1990, and the updated series (1935–1963, 1985–1990, and 1991–2008).

We tested the relationship between temperature and two measures of recruitment using anomalies because we consid-

Fig. 2. (*a*) Comparison of the 3-year average SIO pier surface temperature with the 3-year average 10 m depth reconstructed temperatures from the 2° × 2° grid squares in Fig. 1. (*b*) Difference between the two time series calculated as SIO pier temperature – offshore SCB temperature.



cred that deviations from the mean of the time series would best express the relationship with temperature. Anomalies were calculated by subtracting the mean and dividing by the standard deviation of the entire series. First we used the anomalies of the residuals from the stock recruitment model (SR anomalies) to remove effects SSB on the temperature–recruitment relationship. We then examined the relationship between temperature and reproductive success (R/S, number of age-2 fish/SSB) because it is considered to be more sensitive to environment than is the number of recruits (Wada and Jacobson 1998). Both the SR anomaly and the R/S anomaly remove the effect of stock size on the relationship between numbers of recruits and temperature. The R/S anomaly is more empirical in not assuming any underlying model for the stock–recruit relationship, whereas the SR anomalies are deviations from the fitted SR model. We regressed the recruitment anomalies against both the SIO pier 3-year mean temperatures and the offshore SCB 3-year mean temperatures.

Results

Comparison of SIO pier and offshore temperature trends

Visual inspection of the SIO pier and the offshore SCB temperature time series (Fig. 2*a*) indicates that the SIO pier temperatures have been increasing over the last decades in contrast to the offshore SCB series that did not change noticeably. From 1940 to 1975 3-year means of monthly surface temperatures were 1.5–2 °C higher at the SIO pier compared with the offshore SCB fairly consistently (Fig. 2*b*). Since 1975, the SIO pier temperatures warmed more than the offshore SCB series, and in recent years the difference between the two temperature time series increased to about 3 °C (Fig. 2*b*). The SIO pier and the offshore SCB series are quite similar at high frequencies, and the major difference between them is in the low frequency trends, which have become increasingly different since 1975 (Fig. 2*b*).

SIO pier temperature and Pacific sardine recruitment

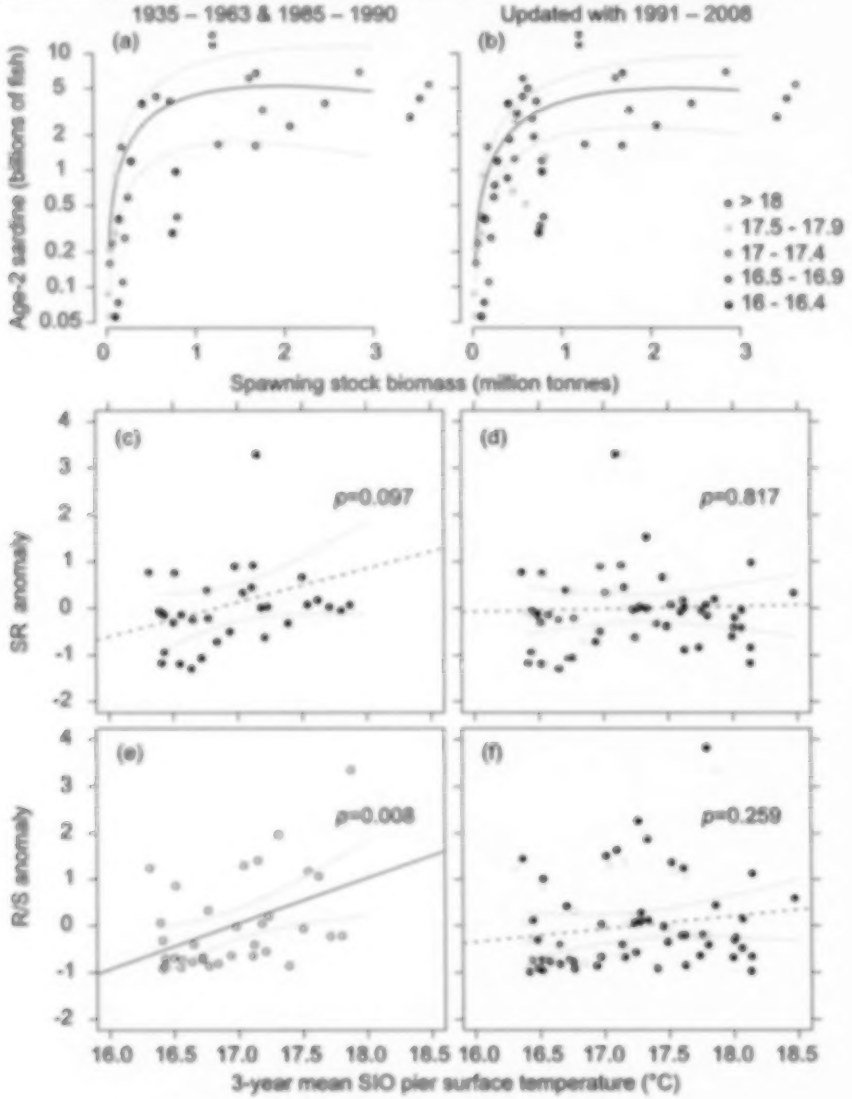
The Ricker model for the early series (Fig. 3*a*) was $R = 7.303 \times 10^3 \mathrm{SSB}\exp^{5.128 \times 10^{-7}\mathrm{SSB}}$ where R represents age-2 recruits and SSB represents spawning stock biomass of sardines. The Ricker model for the updated series (Fig. 3*b*) was $R = 5.834 \times 10^3 \mathrm{SSB}\exp^{4.298 \times 10^{-7}\mathrm{SSB}}$. The model fit both data sets ($p = 0.0017$ for the regression slope and $p = 0.0013$ for the intercept of the early series; $p = 2.03 \times 10^{-5}$ for the slope and $p = 0.00032$ for the intercept of the updated series). Although the stock recruitment relationship was consistent for both time periods, the SIO pier temperatures were cooler in the early period. There were no years where the SIO pier temperatures were higher than 18 °C in the early data (Fig. 3*a*).

Both the SR anomalies and the R/S anomalies were linearly related to 3-year SIO pier temperature in the early years, in agreement with Jacobson and MacCall (1995) (Figs. 3*c* and 3*e*). For both anomalies the relationship broke down when more recent data from 1991 to 2008 were added (Figs. 3*d* and 3*f*). In addition, the linear relationship between SR anomaly and offshore SCB temperatures was weak ($p = 0.156$, adj. $R^2 = 0.021$). There was also no linear relationship between R/S anomalies and offshore SCB temperatures ($p = 0.521$, adj. $R^2 = -0.012$). This indicates that both the SIO pier temperatures and the offshore SCB temperatures are not linearly related to sardine recruitment.

Autocorrelation

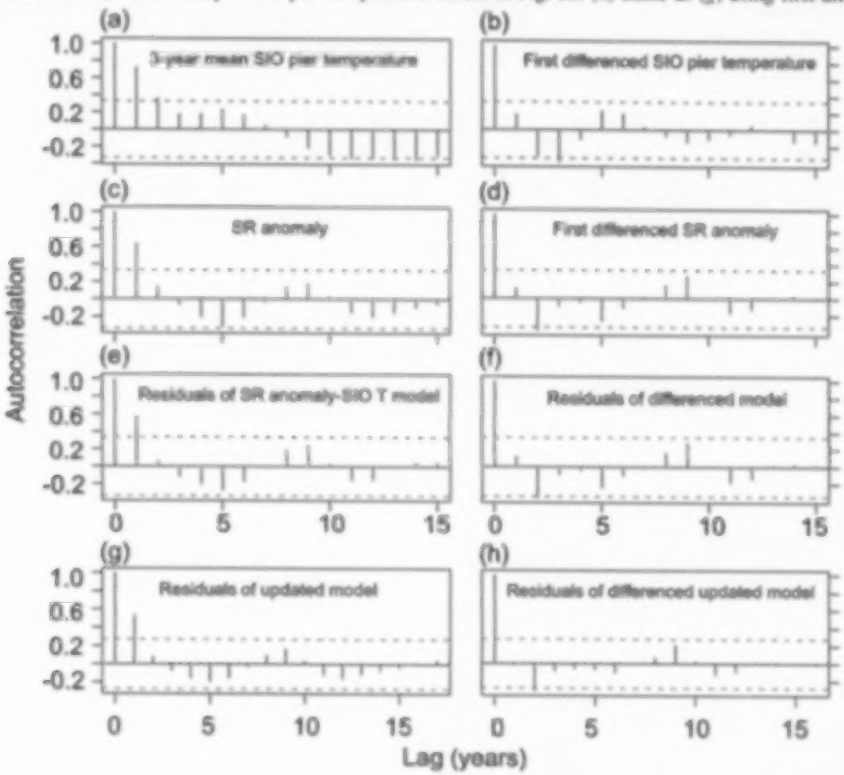
There is an additional problem with the temperature–recruit relationship that affects the significance of the correlation between the variables. Both the 3-year mean SIO pier temperature and the stock–recruit anomaly time series are significantly autocorrelated at a one year lag (Figs. 4*a* and 4*c*). Further, the residuals of the SR anomaly – SIO pier temperature model and the updated version of this model are also significantly autocorrelated (Figs. 4*e* and 4*g*). The autocorrelation in the variables artificially inflates the coefficient of variation and the confidence limits, causing the temperature–recruit relationship to appear stronger than it actually is. This problem is common in relationships between environmental variables and fisheries catches (Drinkwater and Myers 1987) or recruits (Pyper and Peterman 1998). There are several ways of adjusting for the autocorrelation (Pyper and Peterman 1998), but it is common to find that the correlations are no longer significant after adjustments are made (Drinkwater and Myers 1987). The effect of autocorrelation was also observed in the SR anomaly – SIO pier temperature model. While the original relationship

Fig. 3. (*a*) Spawning stock biomass–recruit (age-2 Pacific sardine) relationship using data from Jacobson and MacCall (1995; Table 1, Erratum) for years 1935–1963 and 1985–1990. Black line is a fitted Ricker curve and gray lines are 95% confidence intervals. Data points are color-coded by SIO pier temperature (°C) (*b*) Same as (*a*) updated with data from and Hill et al. (2008; data from 1991–2008). (*c*) Stock–recruit (SR) anomaly calculated from (*a*) in relation to SIO pier temperature for years 1935–1963 and 1985–1990; *p* is the probability that the slope of the linear regression is not different from zero. Grey lines are the 95% confidence intervals (*d*) SR anomaly calculated from (*b*) in relation to updated SIO pier temperature, updated with recent years (1991–2008). (*e*) Recruitment success (R/S) anomaly based on numbers of age-2 sardines in relation to the 3-year mean SIO pier surface temperature using data from Jacobson and MacCall (1995) (1935–1963, 1985–1990). (*f*) Same as (*e*), but updated with recent years (1991–2008). Grey circles are the data from (*e*).



showed a fairly strong, but not a significant correlation (significance of the slope, $p = 0.097$, Fig. 3*c*) when autocorrelation was removed by first differencing the variables (Figs. 4*b* and 4*d*), the correlation all but disappeared, with non-significant slope in the regression ($p = 0.82$). The effect of autocorrelation on the updated SR anomaly – SIO pier temperature model (Fig. 3*d*) was less drastic where significance of the slope coefficient was $p = 0.817$ for the autocorrelated variables and $p = 0.86$ after autocorrelation was removed by differencing. Averaging monthly data over three years changes the pattern of autocorrelation by reducing the seasonal signal (Figs. 5*a* and 5*b*). Averaging to a yearly value reduces but does not eliminate significant autocorrelation (Fig. 5*c*). The sign of the autocorrelation changes in the original temperature series because of the gap between 1963 and 1985 (Fig. 5*d*). The remaining autocorrelation suggests that the original relationship may well have been spurious and was due to the autocorrelation of the variables in the regression.

Fig. 4. (*a*) Autocorrelation of the 3-year mean SIO pier temperature series for 1935–1963 and 1985–1990. Bars extending beyond broken lines indicate significant autocorrelation ($p < 0.05$). (*b*) Same as (*a*) after first differencing the series. (*c*) Autocorrelation of the stock–recruit (SR) anomaly series for 1935–1963 and 1985–1990. (*d*) Same as (*c*) after first differencing the series. (*e*) Autocorrelation of the residuals of the SR anomaly – SIO pier temperature model in Fig. 3*c*. (*f*) Same as (*e*) using first-differenced variables. (*g*) Autocorrelation of the residuals of the updated (1991–2008) SR anomaly – SIO pier temperature model in Fig. 3*d*. (*h*) Same as (*g*) using first-differenced variables.



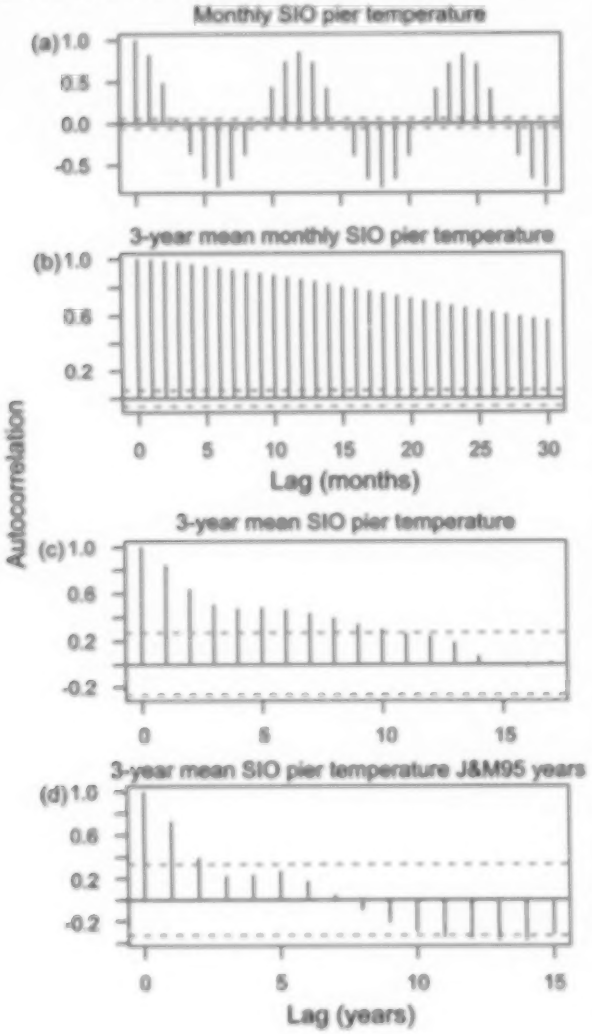
The failure of the temperature–recruit relationship is perhaps to be expected (Myers 1998). That being the case, the basis for the current decision rule in the Coastal Pelagic Species Fishery Management Plan based on the SIO pier temperature index is no longer justifiable, although temperature may affect the growth, fecundity, or later survival of sardines. In addition, we found no relationship between the offshore SCB temperatures and sardine SR anomalies or R/S anomalies, which means that we cannot simply substitute offshore SCB temperatures for the SIO pier temperatures. We need a new index, based on other environmental parameters. A cautionary note might be that any new index should be frequently re-assessed to determine if the underlying relationships can withstand adding new data.

Discussion

Addition of new data causes the temperature–recruit relationship (Jacobson and MacCall 1995) to fail using either the SIO pier temperatures or the offshore temperatures from areas where the sardine currently spawn. Our results show that one of the fundamental relationships (the temperature–recruit relationship) underpinning the stock–recruit-environment relationship does not fit the more recent data. The other fundamental relationship underpinning the original model (the stock–recruit relationship) remains valid when tested with more recent data. Why should this be the case? By matching the early data points to the updated data points it is apparent that many of the more recent recruitment estimates are associated with warmer SIO pier temperatures. In fact, all of the years from 1991 to 2008 except 2001 had 3-year SIO pier temperatures ≥ 17.3 °C. Further, all of the years from 1991 to 2008 except 1998, 2002, 2007 and 2008 had negative SR anomalies, indicating that recruit numbers were lower than the mean after accounting for stock size. After removing the effect of SSB, low recruitment in the warmest years reduced the correlation between between age-2 recruits and temperature, causing the failure of the temperature–recruit relationship. This runs counter to the current theory that warm years are better for sardine recruitment.

The SIO pier temperatures no longer show the same trend as the offshore SCB temperatures where sardine are currently spawning. The explanation for the increasing difference between SIO pier and offshore SCB temperatures is not simple, because the SIO pier is more exposed to waters of tropical origins (e.g., through coastally trapped waves at the surface (Auad and Hendershott 1997) and through the California Undercurrent at deeper levels) while a good deal of the water masses present in the center of the SCB have a

Fig. 5. (*a*) Autocorrelation of the monthly SIO pier temperature series before 3-year averaging. (*b*) Autocorrelation of the same monthly series after averaging. (*c*) Autocorrelation of 3-year mean SIO pier temperature yearly series. (*d*) Autocorrelation of 3-year mean SIO pier temperature yearly series for the years 1935–1963 and 1985–1990 used in Jacobson and MacCall (1995).



sub-arctic origin. Thus, depending on how the sources vary, the SIO pier to offshore SCB gradient will also vary. Warm, saline, low oxygen poleward flows characterize the coastal location of the SIO pier, but the offshore SCB is dominantly exposed to waters with lower temperature and salinity and higher oxygen content. The atmospheric forcing of these two areas is also different as wind speeds are significantly faster over the offshore SCB (Dorman and Winant 2000) than at the SIO pier.

In past sardine assessments based on the CANSAR model (Deriso et al. 1996; Hill et al. 1999), the approach to accounting for environmental effects on recruitment was to use the data in the direct calculation of the expected level of recruitment via an explicit term in the stock–recruit model. The approach currently preferred is to use the environmental data as if it were a survey observation of the recruitment deviation (Methot 2009; Schirripa et al. 2009). The environmental index is treated as if it were a survey of age-0 recruitment abundance because by focusing on the fit to the deviations it removes the effect of spawning stock biomass on recruitment. In this alternative, the recruitment variance (σ_R) would not be changed by the environmental data; instead the environmental data would be used to explain some of the total variability represented by σ_R. Moreover, this approach allows the environmental time series to be input with measurement error.

There is now considerable evidence that relationships between stock, recruitment, and environment may prove to be illusory (Myers 1998). In most cases where statistical relationships have been found between environmental variables and either the biomass of commercially exploited fishes or some index of recruitment, the relationships were not robust when further data were added to the original dataset (Myers 1998). The observation that stock–recruit-environment relationships generally fail has not prevented a burgeoning literature presenting newly discovered relationships. One emergent generalization from Myers (1998) is that stock–recruit-environment relationships tend to be more robust at the margins of a species geographical range. This is presumably because the physiological tolerances of species are approached and so the impact of climate change on environment has greater effect.

Interactions between environmental effects and the impacts of fishing on species biomass or recruitment are also affected by geographical location and species biogeographic range. Because environmental indices may show greater correlations with stock and recruitment at the margins of species range, the effects of environment alone could induce population collapse (Beaugrand and Kirby 2009). In the center of the species range, the effects of environment may be less, and the relative impact of fishing correspondingly greater. It is consequently difficult to develop environmental indices using data over an entire species range. This was shown by Beaugrand and Kirby (2009) where their plankton index was correlated with the decline of Atlantic cod (*Gadus morhua*) in the North Sea, but had low explanatory power for fluctuations of Atlantic cod biomass off Iceland. For the Pacific sardine, the modeled estimates of recruitment are derived from the SCB which is near the center of the species geographic range. We do not have data that would allow us to test stock–recruit-environment relationships at the margins of the range.

The effect of geographical range and the regional differences on the explanatory power of environmental indices is complicated by the evidence that populations and communities are modifying their geographical ranges with climate change (Hsieh et al. 2009). Developing indices necessarily requires time series, but if ranges are shifting latitudinally with climate change, the movement in the margins of the range will tend to obscure the relationship between environmental indices and biomass or recruitment by increasing variability in the relationship.

These considerations; that stock–recruit-environment relationships are strongest at the margins of a species biogeographic range, and that the ranges of populations shift with climate change indicate that stock–recruit-environment relationships are both spatially and temporally dynamic. Recent studies show that the search for stock–recruit-environment relationships that apply over the full spatial domain of a fish species or near the center of the geographic range using long time series of indices are doomed to temporary success at best.

There is some evidence that the most consistent explanatory variables are those that exert both direct effects on physiological tolerances and that affect food web processes such as energy allocation, predator–prey interactions and benthic–pelagic coupling (Kirby and Beaugrand 2009). Temperature is one such variable (Beaugrand and Kirby 2009; Kirby and Beaugrand 2009). Kirby and Beaugrand (2009) used partial correlation and path analysis to reveal consistent correlative relationships between the relative abundance of *Calanus finmarchicus* (an important prey item for larval Atlantic cod), and the biomass and recruitment of Atlantic cod. They interpreted these non-linear correlations as trophic amplification of the effects of temperature through the food web. The observation that the correlations are greatest with a time lag was used to infer that the amplification acts on sensitive life history stages, namely the larval phase of development. Although temperature exerted both direct and food web amplification effects on Atlantic cod larvae, temperature exerts a noisier effect on Pacific sardine. This raises the distinct possibility that the most consistent explanatory variables in stock–recruit-environment relationships is different between species.

What might a new environmental index for Pacific sardine recruitment look like? There have been studies addressing the effect of temperature, salinity, chlorophyll, mesoscale features, advection and wind on the spawning habitat, biomass, growth, survival and recruitment variability of Pacific sardine (Reiss et al. 2008; Rykaczewski and Checkley 2008; MacCall 2009, and references therein), but there are fewer studies on the effects of production, predators and prey. We plan to develop a new environmental index incorporating both physical variables and measures of larval predators and prey to replace the SIO pier temperature index. Given what we know about communities shifting geographic range under climate change and the sensitivity to environmental variability at the edges of a species range, spatial modeling is expected to play an important role in producing a more robust environmental index.

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Environmental drivers of fish functional diversity and composition in the Lower Colorado River Basin

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Abstract: Freshwater conservation efforts require an understanding of how natural and anthropogenic factors shape the present-day biogeography of native and non-native species. This knowledge need is especially acute for imperiled native fishes in the highly modified Lower Colorado River Basin (LCRB), USA. In the present study we employed both a taxonomic and functional approach to explore how natural and human-related environmental drivers shape landscape-scale patterns of fish community composition in the LCRB. Our results showed that hydrologic alteration, watershed land use, and regional climate explained 30.3% and 44.7% of the total variation in fish community taxonomic and functional composition, respectively. Watersheds with greater dam densities and upstream storage capacity supported higher non-native functional diversity, suggesting that dams have provided additional "niche opportunities" for non-native equilibrium life-history strategists by introducing new reservoir habitat and modifying downstream flow and thermal regimes. By contrast, watersheds characterized by greater upstream land protection, lower dam densities, and higher variation in spring and summer precipitation supported fish communities with a strong complement of native species (opportunistic–periodic strategists). In conclusion, our study highlights the utility of a life-history approach to better understand the patterns and processes by which fish communities vary along environmental gradients.

Résumé : Les efforts de conservation en eau douce nécessitent une compréhension de la manière dont les facteurs naturels et anthropiques affectent la biogéographie actuelle des espèces indigènes et non indigènes. Cette connaissance est particulièrement indispensable pour les poissons indigènes en péril dans le bassin versant inférieur fortement modifié du Colorado (LCRB). Nous utilisons dans notre étude à la fois des méthodologies taxonomiques et fonctionnelles pour explorer comment les facteurs environnementaux naturels et anthropiques transforment les patrons de la communauté de poissons à l'échelle du paysage dans le LCBR. Nos résultats montrent que les modifications hydrologiques, l'utilisation des terres du bassin versant et le climat régional expliquent respectivement 30,3 % et 44,7 % de la variation totale de la composition taxonomique et fonctionnelle de la communauté de poissons. Les bassins versants avec une densité plus élevée de barrages et une capacité plus importante de rétention en amont contiennent une diversité fonctionnelle non indigène plus élevée, ce qui laisse croire que les barrages fournissent des « possibilités de niches » pour des espèces non indigènes stratèges d'équilibre en créant de nouveaux habitats de réservoir et en modifiant les régimes de débit et de température en aval. En revanche, les bassins versants caractérisés par une plus grande protection des terres en amont, une densité plus faible de barrages et une variation plus importante des précipitations de printemps et d'été contiennent des communautés de poissons avec une forte composante d'espèces indigènes (stratèges périodiques et opportunistes). En conclusion, notre étude souligne l'utilité d'une approche basée sur les cycles biologiques pour mieux comprendre les patrons et les processus qui expliquent la variation des communautés de poissons le long des gradients environnementaux.

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Introduction

Early explorations of desert ecosystems fueled the perception that these arid environments exhibit depressed species diversity with only modest scientific or conservation value. Further investigations, however, have shown that desert ecosystems often harbor high species endemism that disproportionately contribute to regional diversity and support critical ecological functions (Whitford 2002). Highly dynamic physical conditions and a rich geologic history promoting regional isolation have facilitated the evolution of unique species and life-history strategies in deserts. Conversely, these same ecosystem attributes have made many desert species highly vulnerable to human-induced environmental change, such as increasing agricultural and domestic water demand, habitat loss and fragmentation, introduced invasive species (Williams 1999), thereby qualifying deserts among the most critically imperiled global habitats (Olson and Dinerstein 1998). With over two billion people currently inhabiting arid regions around the world, a central challenge in ecology is to understand the critical linkages between environmental change and biological diversity in these fragile ecosystems (Millennium Ecosystem Assessment 2005).

Streams play a vital role in shaping the hydrological, biogeochemical, and ecological structure and function of desert ecosystems (Sponseller and Fisher 2006; Levick et al. 2008). Episodic patterns in precipitation result in extreme hydrologic regimes that structure the ecological processes and productivity of aquatic and riparian communities and shape species life histories over evolutionary time scales (Lytle and Poff 2004; Naiman et al. 2008). In recent decades, human use of arid system rivers has elevated conflicts between societal and ecosystem needs for water in desert regions around the world. These pressures are particularly acute in the Colorado River — the lifeline of the American Southwest that provides water for human consumption, agriculture, hydroelectricity, and recreation to over 30 million people in the southwestern United States and northwestern Mexico (Fradkin 1981; Carlson and Muth 1989; Paukert et al. 2010). Concurrently, the highly endemic fish fauna of the Lower Colorado River Basin (LCRB) has been detrimentally impacted by these human activities resulting in extensive native species declines in range or abundance over the past century (Miller 1961; Minckley and Deacon 1968; Rinne and Miller 2006). Approximately 25 of the 31 native fish species within the LCRB are federally listed under the US Endangered Species Act (United States Fish and Wildlife Service 1999), and recent research has suggested that many unlisted species are similarly threatened (Fagan et al. 2005*a*; Olden et al. 2008). In contrast with declines in native fishes, the distribution and diversity of many non-native fishes throughout the LCRB have increased rapidly during the same time period (Olden and Poff 2005).

Understanding how natural and anthropogenic factors shape the present-day diversity and composition of fish communities is needed to effectively manage and conserve freshwater ecosystems in the future (Olden et al. 2010). Ecologists traditionally have explored linkages between descriptors of the environment and taxonomic composition of fish faunas, including species occurrence and richness (Jackson et al. 2001). However, a functional perspective using biological traits allows the comparison of species compositions that naturally differ because of biogeographic constraints on regional species pools at larger spatial scales (Schlosser 1990; Poff 1997; Mason et al. 2005). Fish species traits and associated life-history strategies are reflections of the physiological constraints or trade-offs made in response to temporal and spatial variation in abiotic environmental conditions, competition for resources, and predation (Winemiller 2005). By incorporating biological traits into community analyses, one can gain greater mechanistic insight into the environmental drivers of freshwater fish species composition and diversity (e.g., Poff and Allan 1995; Lamouroux et al. 2002; Irz et al. 2007). A functional approach is relevant in the LCRB, where there is increasing interest in clarifying the role of both individual and interactive impacts of natural and human-related drivers in shaping native fish persistence and non-native fish invasions in a highly modified basin.

The objective of the present study was to explore landscape-scale patterns and drivers of fish community composition in the LCRB. First, we examined how the functional diversity of native and non-native fish communities related to hydrologic alteration, land use, regional climate, and watershed physiography. Second, we quantified the differential effects of human- and natural-related factors in explaining patterns of taxonomic versus functional fish community composition. By undertaking a multivariate trait-based examination of species–environment relationships, we aim to provide new insight into the drivers of fish faunal change for an iconic river basin recognized as being among the most threatened globally.

Materials and methods

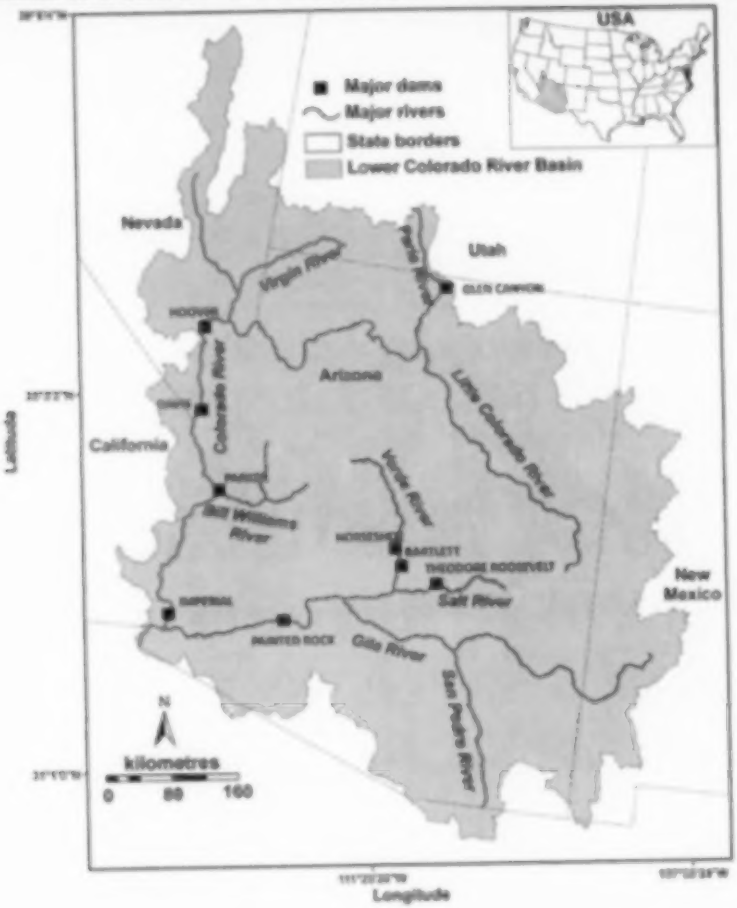
Study area

The LCRB encompasses an area of approximately 362 750 km^2, draining desert and xeric shrublands of the Sonoran Desert and Colorado Plateau and high elevation pinyon–juniper–oak woodlands of the Arizona mountain forests. We defined the boundary of the LCRB as beginning at the base of Glen Canyon Dam and ending at the Gulf of California. The major tributaries of this basin include the Little Colorado, Virgin, Bill Williams, and Gila rivers (Fig. 1). Following the methodology of Sowa et al. (2007) and Hawkins et al. (2000), we divided the LCRB into 386 aquatic ecological system units, hereafter called watersheds, according to abrupt changes in landform, gradient, or stream size. Watershed area ranged from 200 to 1600 km^2, a size considered effective to represent landscape-scale habitat heterogeneity relevant to structuring fish assemblages and provide ecologically meaningful conservation planning units (Sowa et al. 2005; Stagliano 2005).

Fish data

As part of the LCRB Aquatic Gap Analysis Project, we developed a comprehensive database containing >2 500 000 individual fish records for native and non-native fishes covering the period from 1840 to 2006 (Whittier et al. 2006). Records from published, peer-reviewed literature were typically cross-referenced with voucher specimens from museums, and records from agency–institutional reports and other sources of "gray literature" were only used after being

Fig. 1. Map of the Lower Colorado River Basin (LCRB), USA, displaying the major rivers, dams, and basin boundaries.



reviewed for accuracy. Records from the electronic data sets were cross-referenced to site localities and voucher specimens if a record's validity was in question. When a voucher specimen was unavailable, we requested record verification. Records include incidence, identity, and collection information for the complete holdings of major museum collections, numerous smaller holdings, and records from state agencies, peer-reviewed, and gray literature sources. While we recognize the potential limitations associated with analyzing compiled data that was not systematically collected, such as museum-based specimen data (Graham et al. 2004), this database was constructed with a high level of quality control. The size, spatial extent, and temporal scope of this database makes it the most extensive source of fish faunal information for the LCRB and unique among data sets for any major drainage basin in the world.

Our study examined present-day fish faunas by utilizing 1 385 874 fish records collected from 1980 to 2006 for 21 native and 36 non-native species (Table 1); excluded were reintroduction records for native fish and records without location collection data or year of observation. Following Fagan et al. (2002), we considered all collections after 1980 to represent present-day fish assemblages — a robust breakpoint for comparing distributional trends over time in the LCRB (Fagan et al. 2005*b*). In addition to Fagan et al. (2005*b*), Olden and Poff (2005) examined temporal patterns of native and non-native fish distributions in the LCRB by decade and identified 1981 as a breakpoint representing extant species ranges. These modern records in the data set are almost exclusively the result of intensive efforts by federal or state agencies to determine species' complete distributions prior to listing decisions under the US or Mexican Endangered Species Acts (Fagan et al. 2002). In total, our analysis was based on a selection of 159 watersheds after removing watersheds with no fish records or low sampling effort.

Functional attributes were characterized for each fish species by utilizing a database of 14 biological and ecological traits (i.e., morphological, behavioral, trophic, and life history) for the Colorado River Basin assembled by Olden et al. (2006). These traits were chosen to reflect the major ecological strategies exhibited by fishes in this region (Table 2). Trait assignments were constructed using a multitiered data collection protocol, utilizing comprehensive texts of state fish faunas, primary literature, state agency reports, university reports, and graduate theses. Electronic databases and expert knowledge were used to supplement the literature-

Table 1. Freshwater native (n = 21) and non-native (n = 36) fish of the Lower Colorado River Basin.

Native species, common name	Code	Non-native species, common name	Code
Catostomidae		**Catostomidae**	
Catostomus clarkii, desert sucker	CACL	*Ictiobus cyprinellus*, bigmouth buffalo	ICCY
Catostomus discobolus, bluehead sucker	CADI	**Centrarchidae**	
Catostomus insignis, Sonora sucker	CAIN	*Ambloplites rupestris*, rock bass	AMRU
Catostomus latipinnis, flannelmouth sucker	CALA	*Lepomis cyanellus*, green sunfish	LECY
Xyrauchen texanus, razorback sucker	XYTE	*Lepomis macrochirus*, bluegill	LEMA
Cyprinidae		*Lepomis microlophus*, redear sunfish	LEMI
Agosia chrysogaster, longfin dace	AGCH	*Micropterus dolomieu*, smallmouth bass	MIDO
Gila cypha, humpback chub	GICY	*Micropterus salmoides*, largemouth bass	MISA
Gila elegans, bonytail	GIEL	*Pomoxis nigromaculatus*, black crappie	PONI
Gila intermedia, Gila chub	GIIN	**Cichlidae**	
Gila nigra, headwater chub	GINI	*Oreochromis aureus*, blue tilapia	ORAU
Gila robusta, roundtail chub	GIRO	*Oreochromis mossambicus*, Mozambique tilapia	ORMO
Lepidomeda mollispinis, Virgin spinedace	LEMO	*Tilapia zillii*, redbelly tilapia	TIZI
Lepidomeda vittata, Little Colorado spinedace	LEVI	**Clupeidae**	
Meda fulgida, spikedace	MEFU	*Dorosoma petenense*, threadfin shad	DOPE
Plagopterus argentissimus, woundfin	PLAR	**Cyprinidae**	
Rhinichthys cobitis, loach minnow	RHCO	*Carassius auratus*, goldfish	CAAU
Rhinichthys osculus, speckled dace	RHOS	*Ctenopharyngodon idella*, grass carp	CTID
Cyprinodontidae		*Cyprinella lutrensis*, red shiner	CYLU
Cyprinodon macularius, desert pupfish	CYMA	*Cyprinus carpio*, common carp	CYCA
Poeciliidae		*Notemigonus crysoleucas*, golden shiner	NOCR
Poeciliopsis occidentalis, Gila topminnow	POOCOC	*Pimephales promelas*, fathead minnow	PIPR
Salmonidae		*Richardsonius balteatus*, redside shiner	RIBA
Oncorhynchus gilae apache, Apache trout	ONGIAP	**Esocidae**	
Oncorhynchus gilae, Gila trout	ONGIGI	*Esox lucius*, northern pike	ESLU
		Fundulidae	
		Fundulus zebrinus, plains killifish	FUZE
		Ictaluridae	
		Ameiurus melas, black bullhead	AMME
		Ameiurus natalis, yellow bullhead	AMNA
		Ictalurus punctatus, channel catfish	ICPU
		Pylodictis olivaris, flathead catfish	PYOL
		Moronidae	
		Morone mississippiensis, yellow bass	MOMI
		Morone saxatilis, striped bass	MOSA
		Percidae	
		Perca flavescens, yellow perch	PEFL
		Sander vitreus, walleye	SAVI
		Poecillidae	
		Gambusia affinis, western mosquitofish	GAAF
		Poecilia latipinna, sailfin molly	POLA
		Salmonidae	
		Oncorhynchus clarkii, cutthroat trout	ONCL
		Oncorhynchus mykiss, rainbow trout	ONMY
		Salmo trutta, brown trout	SATR
		Salvelinus fontinalis, brook trout	SAFO
		Thymallus arcticus, Arctic grayling	THAR

based trait assignments in a limited number of cases. Trait values were recorded based on research conducted in the LCRB or the closest geographic region when possible to account for interdemic variation in biological traits.

Environmental characteristics

We quantified 17 environmental characteristics associated with natural and human-related landscape features based on their demonstrated importance in shaping patterns of fish species occurrence in arid and semiarid ecosystems. Variables were either calculated for the watershed or included the upstream (US) contributing basin and were grouped into categories describing hydrologic alteration by dams, land use and watershed physiography, and regional climate (numbered sequentially below). Variables describing hydrologic alteration included (1) US dam density (no.·km^{-2}); (2) US dam storage capacity (m^3); (3) US reservoir surface area (km^2); (4) US canal–ditch density (m·km^{-2}); and

Table 2. The functional attributes of each fish species examined in this study.

Category	Trait	Description and (or) units	Abbrev.
Morphology	Maximum total body length*	Maximum total body length (cm)	MS
	Swim factor*	Ratio of minimum depth of the caudal peduncle to the maximum depth of the caudal fin	SF
Behavior	Water temperature preference	Cold (10–17 °C), cool (18–26 °C), or warm (>26 °C) based on species distributions and physiological optima	TP
	Substrate preference	Rubble–cobble–gravel, sand, silt–mud, or general	SP
	Fluvial dependence	Relying or not relying on flowing waters for completing life cycle, e.g., flow required for feeding or reproduction	FD
	Flow preference	Slow, slow–moderate, moderate, moderate–fast, or fast current velocity	FP
Trophic	Trophic guild	Omnivore (approximately <5% plant matter), invertivore, invertivore–piscivore, piscivore, and herbivore–detritivore (approximately >25% plant matter) based on published adult diet analyses	TG
Life history	Longevity*	Maximum potential life span (years)	LM
	Age at maturation*	Age at maturation (years)	AM
	Length at maturation*	Length at maturation (cm)	LM
	Fecundity*	Total number of eggs or offspring per breeding season	F
	Egg size*	Mean diameter of mature oocytes (mm)	ES
	Parental care	Total energetic contribution of parents to offspring	PC
	Reproductive guild	Bearers (internal), guarders (nest spawners or substratum choosers), or non-guarders (brood hiders or open substratum spawners)	RG

*Continuous trait characteristics that were converted into categorical data for functional community analysis included maximum total body length (1: 0–400 mm; 2: 401–800 mm; 3: >800 mm); swim factor (1: 0–4; 2: >4); longevity (1: 0–5 years; 2: 5–10 years; 3: >10 years); age at maturation (1: 0–1 years; 2: 1–2 years; 3: >2 years); length at maturation (1: 0–100 cm; 2: 101–200 cm; 3: 201–300 cm; 4: >300 cm); fecundity (1: 0–4; 2: >4); and egg size (1: 0–1.5 mm; 2: >1.5–3 mm; 3: >3 mm). Taxonomic and functional analyses using canonical correspondence analysis (CCA) and partial CCA (pCCA) were performed for the full species data set, native species only, and non-native species only.

(5) downstream distance (km) from the tail-water boundary for each watershed to the nearest dam to represent proximity to lentic habitat. Data sources used were the National Inventory of Dams (United States Army Corps of Engineers 2007) and the National Hydrography Dataset (United States Geological Survey 2007). Land use and watershed physiographic variables included (6) US agricultural land use (%); (7) road density ($m \cdot km^{-2}$); (8) US land in urban development (%); (9) US natural land dominated by barren rock, forest, or scrub brush (%); (10) US land with permanent protection from human development according to a regional management plan (%); (11) canyon habitat (km) calculated as the total length of stream segments dominated by canyon habitat (Whittier et al. 2006); and (12) watershed surface area (km^2). Data sources were the National Land Cover Database (Multi-Resolution Land Characteristics Consortium 2001), Southwest Regional Gap Analysis Project Stewardship Data (United States Geological Survey 2005), and the National Elevation Dataset (United States Geological Survey 2004). Variables describing regional climatic conditions included (13) mean US annual precipitation (mm); (14) variation in winter precipitation calculated as the coefficient of variance (CV) in monthly US precipitation for November to February; (15) variation in spring precipitation (March to April); (16) variation in summer precipitation (May to August); and (17) mean annual temperature (°C) calculated as the midpoint of maximum and minimum air temperatures recorded across each watershed. All precipitation variables were based on records from 1971 to 2000. Data source was Climate Source (United States Department of Agriculture – National Resources Conservation Service 2007).

Additional variables were originally considered but were removed because they were highly correlated with the variables detailed above. We assessed variable collinearity by computing the variance inflation factor (VIF), where VIF values greater than 5 are considered to be indicative of high collinearity (Menard 1995; O'Brien 2007). None of our environmental variables had VIF values greater than this conservative threshold, indicating an acceptable level of collinearity in subsequent analyses. We tested for spatial dependency among our environmental variables in geographic space by regressing the pairwise similarity in environmental characteristics between watersheds (based on Euclidean distance) against the geographical distance separating them (according to Euclidean distance between centroids of each watershed) using a Mantel test. We found very little spatial structure in our environmental variables among watersheds ($r = 0.043$, $P = 0.075$), suggesting that watersheds could be treated as independent observations in subsequent statistical analyses.

Statistical analysis

Indices of taxonomic and functional diversity

Functional diversity can be described by a number of indices (see Petchey and Gaston 2006), including the three primary components of functional richness, functional evenness, and functional divergence (Mason et al. 2005; Villéger et al. 2008). These functional indices are measured on continuous scales and describe independent aspects of trait diversity in community space. Functional richness measures the amount of trait space or volume occupied by the species of a community; functional evenness describes the uniformity of species (or their abundance) that comprise a community in trait space; and functional divergence measures the degree that abundance-weighted functional differences are maximized in niche space among the species within a community.

We quantified species functional richness and evenness

for watersheds using 14 species traits following Villéger et al. (2008); functional divergence was not computed because reasonable estimates of species abundance at the watershed scale could not be estimated with confidence. The functional richness index value for each watershed was assessed using the Quickhull algorithm (Barber et al. 1996), which calculates the minimum convex hull that includes all the species considered in the multivariate trait space. Functional richness values have no upper limit because they quantify the absolute volume represented by the community's suite of traits in each watershed. The functional evenness index value was calculated using a minimum spanning tree method linking all the trait points in multidimensional space with a minimum sum of total tree branch lengths (Villéger et al. 2008). Functional evenness values are constrained between 0 and 1, with low values representing a high proportion of the species trait occurrence concentrated within a narrow range of the functional trait gradient and high values representing evenly spaced species traits throughout the occupied functional space.

To calculate functional richness and evenness, we first summarized similarities in species' trait characteristics by conducting a principal coordinate analysis (PCoA) on the species-by-trait matrix. PCoA is a statistical methodology to explore similarities by optimally representing the variability of a multidimensional data matrix (species-by-trait matrix) in reduced ordination space (Legendre and Legendre 1998). A species dissimilarity matrix based on the 14 traits was calculated using Gower's coefficient, a metric able to accommodate nominal, ordinal, and continuous data (Pavoine et al. 2009). The first two principal coordinate axes each explained a significant proportion of the total variation in trait community composition (30% and 19%) based on the randomized broken-stick method and $\alpha = 0.05$ (Jackson 1993), and both axes were used to calculate the functional richness and evenness values for each watershed. We calculated both native and non-native functional richness and evenness indices individually for our functional diversity analyses, but because of species richness constraints associated with the functional index calculations, only watersheds that had four or more native species (97 watersheds) or non-native species (126 watersheds) could be examined.

Diversity–environment relationships

We used general linear modeling to model native and non-native functional richness and evenness (separately) as a function of environmental factors describing watershed physiography, hydrologic alteration, land use, and climate. Taxonomic species richness was also included in some models, specifically native richness for non-native models, and vice versa. Our analysis was designed to test the relative importance of different subsets of these factors for predicting native and non-native fish diversity (richness and evenness) using an information-theoretic approach to model selection (Anderson and Burnham 2002). We considered 24 candidate models (Appendix A), including a global model with all 17 environmental variables (model 1), models containing single descriptors of environmental variation (hydrologic alteration: model 2; land use: models 3–6; and climate: models 6–9), models depicting the role of riverine fragmentation (model 10), natural physiographic basin features (model 11), and overall descriptors of human disturbance (models 12–13). Lastly, we examined a series of candidate models derived from the literature reflecting combinations of variables that have been identified as being important in shaping fish community composition in the southwestern USA (models 14–24). These literature-derived models are based on the findings of Moyle and Light (1996), Gido and Brown (1999), Meador et al. (2003), Marchetti et al. (2004), Propst and Gido (2004), Rinne and Miller (2006), Light and Marchetti (2007), and Propst et al. (2008).

Regression diagnostics from the global model indicated a reasonable statistical fit with no significant deviations from the parametric assumptions. Consequently, we ranked our 24 candidate models using Akaike's information criterion (AIC) to evaluate the relative support (given the data) for each model. We also calculated the difference between the best approximating model value and all remaining model values (Anderson and Burnham 2002, ΔAIC_i). Maximum log-likelihood estimates and Akaike weights (w_i), representing the relative likelihood of a model against all candidate models, were calculated for every model. Evidence ratios were computed to quantify the relative support for each model by dividing the w_i of the best approximating model by the w_i for each individual model. For the best approximating model, w_i values and standardized regression coefficients (d_i) for each environmental factor were calculated to assess variable importance (Anderson and Burnham 2002). We calculated the cumulative Akaike weights (Σw_i) for the top five supported models to estimate overall relative importance of the model. Additionally, we calculated cumulative weights based all models found in 95% confidence intervals of all models. The results did not differ substantially between the two approaches, so we present the results from the latter. All model analysis was performed using R 2.51 utilizing existing scripts in the Ape library (R Development Core Team 2007).

Community–environment relationships

We used canonical correspondence analysis (CCA) (ter Braak 1986) to assess the relationship between fish community composition and environmental characteristics at the watershed scale. In our analysis, CCA allows for the ordination of the fish community data (by reciprocal averaging) to be constrained by a multiple regression on its relationships to the environmental variables. CCA is robust when species' responses to the underlying environmental gradients are unimodal and important predictor variables have been measured and are relatively free from stochastic noise (Gauch 1982; Palmer 1993). We selected CCA because an analysis based on detrended correspondence analysis indicated that, on average, the species (gradient level = 2.7) and the traits (gradient level = 3.7) showed unimodal responses along the environmental gradients (Gauch 1982). Contributions of each environmental variable in the CCA were tested for significance using a Monte Carlo test with 4999 permutations, and only those that were statistically significant at $\alpha = 0.05$ were depicted on the ordination biplots. Distributional properties of the variables were examined and resulted in the $\log(x)$ transformation of watershed area, annual precipitation, and annual temperature; the $\log(x + 1)$ transformation of dam density, dam storage, distance to downstream dam,

reservoir surface area, canal density, and road density; and the arcsine (square-root x) transformation of land use variables expressed as proportions (agriculture, urban, natural, and protected).

Partial CCA (pCCA), a modified canonical ordination technique, is an appropriate method for decomposing species–environment relationships via variance partitioning (ter Braak 1988; Borcard et al. 1992). We used pCCA to partition the total community variance explained by the different sets of environmental variables into their independent (pure) and confounded (intercorrelated) components, thus enabling us to assess the relative importance of hydrologic alteration, land use, and climate in explaining patterns in functional and taxonomic community composition of the native and non-native species pools. Land use was further decomposed into the variation explained by variables of human land use (agriculture, urban, road density) versus natural land use (natural land, protected, canyon habitat, and watershed area). We examined both independent and confounded variation explained by each category of variables.

To assess the relationship between functional community composition and environmental drivers, we calculated a watershed-by-trait matrix by multiplying the watershed-by-species matrix by the species-by-trait matrix and dividing by the watershed-by-richness column vector. To accomplish this, we first were required to categorize the continuous traits (e.g., body length) into discrete trait states (see Table 2), which after combining with the raw categorical traits (e.g., trophic guild, reproductive guild) enabled the creation of a species-by-trait state presence–absence matrix. The end result was a watershed-by-trait matrix containing values that represented the proportion of species in each watershed exhibiting each trait state.

Results

Geographic patterns of taxonomic and functional diversity

Native and non-native fishes showed notable spatial patterns of taxonomic and functional diversity. Native taxonomic diversity was greatest in the main stem and tributaries of the Upper Gila River, Verde River, Salt River, and portions of the Upper Virgin River (Fig. 2*a*). Patterns of non-native taxonomic diversity mirrored the native fish fauna, but with relatively higher richness in the main stem Colorado River between Lake Powell (Glen Canyon Dam) and Lake Mead (Hoover Dam) and downstream from Imperial Dam (close to the confluence with the Gila River) and relatively lower richness in the Upper Gila River Basin (Fig. 2*b*). Mean native and non-native species richness throughout the basin was four and seven species per watershed, respectively.

Geographic patterns of functional richness were more concentrated compared with taxonomic richness. High levels of native functional richness were mainly observed in the Verde River, Upper Gila River, and Upper Santa Cruz River (Fig. 2*c*), whereas non-native functional richness was elevated in the main stem Colorado River between Lake Powell and Lake Mead (Fig. 2*d*). The mean functional richness of the native community watersheds (2.1) was lower than the mean functional richness of the non-native community watersheds (3.1) for all watersheds analyzed. By contrast, the mean functional evenness of native communities (0.78) exceeded the functional evenness of non-native communities (0.70).

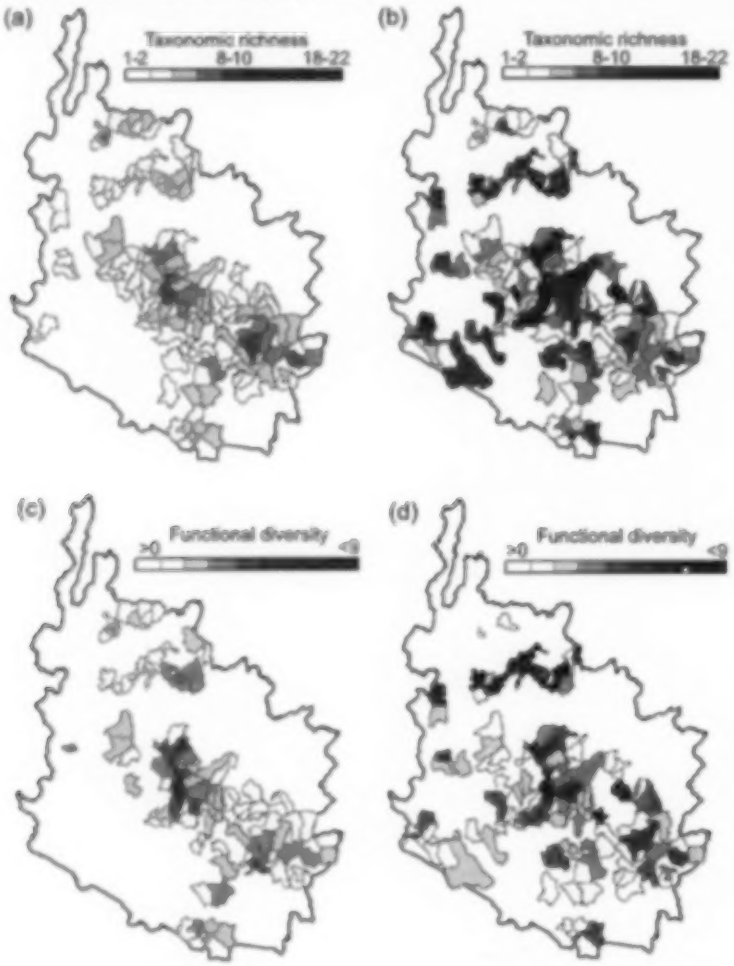
We found a significant positive relationship between taxonomic richness and functional richness ($r^2 = 0.638$, $P < 0.001$, $n = 159$), but only a weak association between taxonomic richness and functional evenness ($r^2 = 0.041$, $P = 0.101$, $n = 159$). As expected, the positive relationship between taxonomic and functional richness occurred because additional species are likely to introduce additional traits into a community.

Environmental drivers of native and non-native functional diversity

The most supportive model for native functional richness was designed from Propst et al. (2008; model 20) and incorporated watershed area; available canyon habitat; annual precipitation; variation in winter, spring, and summer precipitation; and non-native species taxonomic richness (Table 3). This model was four times more likely given the data than the next most competitive model. According to the top model, all of the aforementioned variables were positively related to native functional richness, with the exception of the negative influence of variation in spring precipitation (Tables 3, 4). Cumulative Akaike weights (Σw_i) based on the top five supported models demonstrate that watershed area, non-native taxonomic richness, canyon density, and all seasonal measures of variation in precipitation were the most predictive of native functional richness (Table 4). The model with the most support in describing the native functional evenness was drawn from Marchetti et al. (2004; model 18) and included canal density, urban development in the watershed, annual precipitation, and non-native taxonomic richness (Table 3). Functional evenness of the native communities was negatively related to all these variables, with the exception of canal density. This model had no close competitors, and the cumulative Akaike weights across the top five models supported the overwhelming importance of the four variables listed above.

The most supportive model for non-native functional richness was based on Gido and Brown (1999; model 15) and showed that increased non-native functional richness was related to increased dam density and dam storage capacity, watershed area, and native species taxonomic richness (Tables 3, 4). A variant of this model including canyon habitat (positive effect), annual precipitation (positive effect), and seasonal (CV) precipitation (negative effect) was also highly competitive (Rinne and Miller 2006; model 23), as well as the same model but including canal density (negative effect) (Marchetti et al. 2004; model 19). Model 15 was 1.5 times more likely given the data than the next most competitive model, but was clearly more parsimonious than the latter, having five less parameters. Watershed area, native taxonomic richness, dam storage capacity, and dam density exhibited the highest cumulative Akaike weights across the top five supported models (Table 4). The most competitive model for predicting non-native functional evenness was model 22 (based on Propst and Gido 2004), which included watershed area, annual precipitation, variation in spring and summer precipitation, and native taxonomic richness

Fig. 2. (*a*) Native and (*b*) non-native taxonomic richness and (*c*) native and (*d*) non-native functional richness for watersheds in the Lower Colorado River Basin (LCRB). Taxonomic richness ranged from 1 to 22 species, and functional diversity ranged from 0 to 9 functional volume units. Refer to Fig. 1 for location of dams and major rivers.



(Table 3). This model was 3.4 times more likely given the data than the next most competitive model, and all of the variables were positively related to evenness except for watershed area (Table 4). Cumulative Akaike weights for the top five supported models illustrated the importance of watershed area, variation in spring and summer precipitation, and annual precipitation. Among the top model competitors were three models incorporating land use disturbance (models 6, 7, and 8).

Environmental drivers of native and non-native functional composition

Environmental factors describing hydrologic alteration, watershed physiography – land use, and climate explained 30.3% of the total variation in fish taxonomic composition (native and non-native together), with the first two canonical axes explaining 14.4% of the variation (both $P < 0.05$ based on Monte Carlo simulation). In general, native species occupy the extreme positions in the lower right and upper left quadrants of the taxonomic ordination, suggesting that watersheds associated with greater upstream land protection and higher variation in spring and summer precipitation (representing before and after spawning period for native fishes, respectively) are characterized by a complement of native species (Figs. 3*a*, 3*b*, 3*c*). Native species associated with watersheds with higher proportion of protected lands include Virgin River spinedace, woundfin, bluehead sucker, flannelmouth sucker, and humpback chub, and species associated with more variable precipitation regimes include Gila topminnow, longfin dace, headwater chub, Gila chub and Sonora sucker (scientific names provided in Table 1). The upper right quadrant of the taxonomic ordination contained watersheds characterized by a mixture of predominantly native trout (Apache trout, Gila trout) associated with greater availability of canyon habitat and non-native trout (rainbow trout, brook trout, cutthroat trout) associated with higher dam density (Figs. 3*a*, 3*b*). The lower left quadrant of the taxonomic ordination is populated with non-native species-

Table 3. Summary of the model selection statistics for predicting fish community functional diversity indices for native and non-native species.

Model	K	AIC	Δ_i	w_i	Evidence ratio	Model	K	AIC	Δ_i	w_i	Evidence ratio
Native richness						**Non-native richness**					
20	9	278.6	0.0	0.699	1.0	15	7	496.3	0.0	0.405	1.0
24	8	281.3	2.7	0.175	4.0	23	12	497.2	0.8	0.265	1.5
23	12	282.7	4.1	0.088	8.0	19	8	498.3	2.0	0.149	2.7
8	6	286.8	8.2	0.011	61.1	21	9	498.9	2.5	0.113	3.6
7	7	287.3	8.7	0.009	79.2	16	7	501.6	5.3	0.029	14.0
Native evenness						**Non-native evenness**					
18	6	–221.4	0.0	0.538	1.0	22	7	–227.0	0.0	0.495	1.0
19	8	–218.8	2.6	0.148	3.6	8	6	–224.5	2.5	0.144	3.4
14	11	–218.7	2.7	0.141	3.8	20	9	–223.8	3.1	0.103	4.8
15	7	–217.6	3.8	0.080	6.7	6	8	–223.7	3.3	0.095	5.2
21	9	–217.2	4.2	0.067	8.0	7	7	–223.3	3.7	0.078	6.3

Note: All models displayed were the top five models for each functional index analyses. The evidence ratio is calculated as the Akaike weight of the top model divided by the Akaike weight of each model for each functional analysis. Δ_i (AIC difference) is the difference between the top performing model AIC value and each individual model AIC value within each function analysis. w_i is the Akaike weight calculated as the ratio of each model's likelihood to the sum of all the other model likelihoods for each functional analysis.

Table 4. Summary of the cumulative Akaike weights (Σw_i) for the five top ranked models each functional group and standardized regression coefficients ($\hat{a}_i$) for each best fitting model.

	Functional richness				Functional evenness			
	Native		Non-native		Native		Non-native	
Model variables	Σw_i	$\hat{a}_i$	Σw_i	$\hat{a}_i$	Σw_i	$\hat{a}_i$	Σw_i	$\hat{a}_i$
Hydrologic alteration								
Dam density	0.033		0.932	–0.304	0.436			
Dam storage	0.033		0.961	0.454	0.436			
Distance to down dam								
Reservoir surface area			0.029					
Canal density			0.262		0.893	0.432		
Land use and physiography								
Agriculture	0.065				0.141			
Road density								
Urbanization	0.065		0.113		0.745	–0.154		
Natural								
Protected								
Canyon	0.921	0.197	0.265				0.103	
Watershed area	0.990	0.153	0.961	0.056	0.436		0.916	–0.145
Climate								
Annual precipitation	0.358	0.049	0.294		0.678	–0.359	0.669	0.178
Winter precipitation (CV)	0.921	0.483	0.265				0.420	
Spring precipitation (CV)	0.925	–0.133	0.265				0.916	0.172
Summer precipitation (CV)	0.925	0.285	0.265				0.916	0.276
Watershed annual temperature	0.065						0.095	
Taxonomic richness*	0.986	0.336	0.961	0.456	0.974	–0.136	0.599	0.203

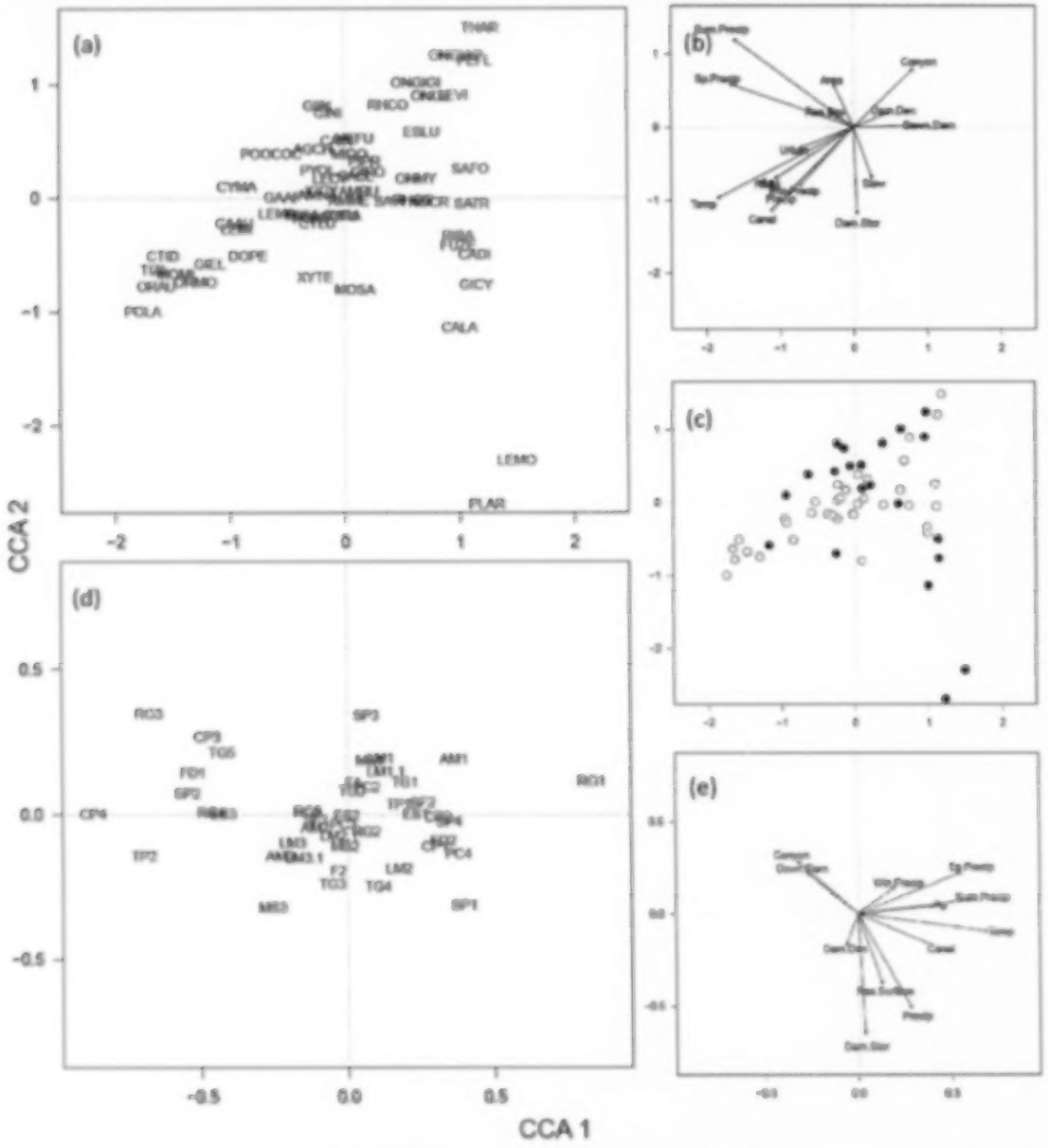
Note: Σw_i is the cumulative Akaike weight for each column calculated by summing the Akaike weights for each of the top five models by group. $\hat{a}_i$ is the standardized regression coefficient for each variable in the best fitting model in each functional analysis.

*Native taxonomic richness was used in the non-native analysis and vice versa.

dominated watersheds characterized by high canal and road densities, high urban land use and agriculture, and warmer and wetter climates (Figs. 3*a*, 3*b*).

Results from the variance partitioning suggest that the major drivers of taxonomic composition differ between native and non-native species. Of the 34% total variation in native species composition described by the suite of environmental variables, regional climate explained the greatest relative amount (54%) of variation compared with hydrologic alteration and watershed physiography – land use (Fig. 4*a*). The same trend was evident for non-native species composition (28.6% of the total variation explained); how-

Fig. 3. Ordination plots resulting from a taxonomic and functional canonical correspondence analysis: (*a*) fish species biplot where letter codes refer to species (Table 1); (*b*) eigenvector plot of environmental characteristics from forward stepwise selection of variables in the taxonomic analysis; (*c*) fish species biplot where solid circles represent native species and open circles represent non-native species; (*d*) trait biplot where letter codes represent the percentage a trait was represented in sites (see Table 2 for letter and number codes); (*e*) eigenvector plot of environmental characteristics from forward stepwise selection of variables in the functional analysis.



ever, climate accounted for a relatively smaller amount (41%) of the variation explained. Variables describing human-related activities (agricultural, urban, road density) explained a greater percentage of variation in non-native communities compared with native communities, whereas the opposite trend was true for variables depicting more natural physical characteristics (natural land use, canyon, watershed area) (Fig. 4*a*).

Factors describing the environmental categories of hydrologic alteration, watershed physiography – land use, and climate explained 44.7% of the total variation in fish functional composition (native and non-native together), with the first two canonical axes explaining 36.0% of the variation (both $P < 0.05$ based on Monte Carlo simulation), which was about 14% more than the taxonomic variation explained by the same set of variables. The left side of the or-

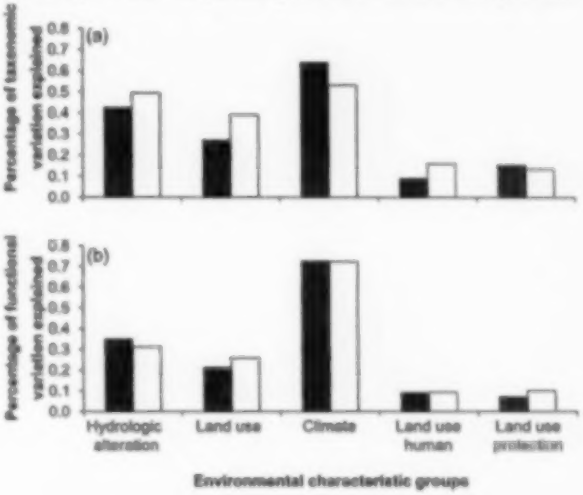
dination represents fish faunas characterized by biological traits describing large body size (MaxS3), older age at maturation (AM3), reproductive guilds representing low parental care (RG3-5), herbivore–detritivore feeding behavior (TG5), preference for fast currents (CP3-4), and fluvial dependence (FD1) (Fig. 3*d*). This suite of traits is indicative of a periodic life-history strategy and is more likely to occur in watersheds with available canyon habitat and increased upstream distance from reservoirs (Fig. 3*e*). The upper right side of the ordination (positive CCA1 and CCA2 scores) is populated by watersheds characterized by species exhibiting small body size (MaxS1), early age at maturation (AM1), low parental care, (PC1), and short life span (Long1) (Fig. 3*d*): a suite of traits that generally define an opportunistic life-history strategy. These watersheds were characterized by increased variation in winter, spring, and summer precipitation (Fig. 3*e*). The lower right ordination space (positive CCA1 and negative CCA2 scores) represents fish faunas represented by biological traits describing moderate life span (Long2), high parental care (PC4), ecological traits describing preference for slow currents (CP1), and a lack of fluvial dependence for life-cycle completion (FD2) (Fig. 3*d*). These trait states are indicative of an equilibrium life-history strategy and were more likely to occur in watersheds with increased canal density, dam reservoir surface area and volume, greater annual air temperatures, and precipitation (Fig. 3*e*).

The amount of variation in functional composition explained by the environmental variables varied considerably less compared with patterns in taxonomic composition. Environmental factors described 38% and 37% of the variation in native and non-native community functional composition, respectively, where regional climate explained the greatest amount of variation followed by hydrologic alteration and watershed physiography – land use (Fig. 4*b*). Categorical differences in the variation explained in native and non-native composition was substantially smaller for functional attributes compared with taxonomy (Fig. 4*a*).

Discussion

Our study explored the patterns and environmental drivers of fish community composition and diversity in the highly modified Lower Colorado River Basin using a combined taxonomic and functional approach. Our results suggest that natural and human-related environmental factors have differentially shaped native and non-native fish communities at the watershed scale. Functional richness of native communities was greater in watersheds characterized by more variable winter and summer precipitation regimes and more stable spring precipitation conditions. The spawning chronology of native species throughout the arid Southwest, in large part, explains this pattern. Larval production for many native species typically peaks during spring runoff, where juvenile fish are believed to have optimal rearing conditions in less variable flow (Gido and Propst 1999; Brown and Ford 2002), and fluctuating summer flows provide juveniles access to critical backwater habitats (Minckley et al. 2003). In our study, seasonal precipitation variation was clearly linked to native communities' functional richness, indicating that natural flow regime variability (a direct result of over-

Fig. 4. Relative independent taxonomic and functional variation explained by hydrologic, land use, and climate variable groups, including subgroups of land use variables associated with human disturbance (land use human) and land use variables associated with natural and (or) protection status (land use protection). Solid bars represent native fishes; open bars represent non-native fishes. Results are presented as a percentage of the total variation explained.



land flow caused by precipitation events) is an important part of the habitat template for native species and can be considered vital for sustaining native species trait diversity at the community scale. Interestingly, variables describing hydrologic alteration and fragmentation by dams were not strong predictors of native functional diversity despite the substantial evidence that dams decouple rivers from their typical seasonal flow patterns (Graf 1999) and negatively impact fluvial-dependent native fishes (Haxton and Findlay 2008). Our results may be related to the spatial grain (i.e., watershed) and numeric resolution (i.e., species presence–absence) of our analysis.

Life-history theory offers a promising path to understanding the patterns and processes by which fish community properties vary along environmental gradients (Winemiller 2005). By comparing trends in native and non-native species distributions among fish life-history strategies in the LCRB, Olden et al. (2006) postulated that century-long modifications in flow variability have likely promoted the spread of non-native equilibrium strategies (favored in constant environments) while leading to greater distributional declines of native species located along the periodic–opportunistic continuum (strategies favored in more unpredictable and variable environments). We explored this reasoning in our study by examining associations between the life-history composition of watersheds and both natural and anthropogenic descriptors of the riverine environments. Watersheds dominated by native opportunistic strategists were characterized by low dam density and seasonally variable precipitation, indicating that unregulated watersheds are indeed more likely to support more native-rich fish communities. These watersheds, particularly tributaries off the main stem Colorado River, which experience extreme seasonal droughts as

well as intense overland flows in response to monsoonal rain events, have become rare in the highly dammed LCRB (Fradkin 1981). With a majority of native species possessing traits defining opportunistic–periodic life histories, maintaining natural flow variability as well as mitigating the impact of dams via water release based on natural flow patterns (environmental flows) may be paramount to maintaining the overall functional diversity of native communities. At a taxonomic level, Propst and Gido (2004) displayed that flow regimes mimicking natural conditions enhanced native fish recruitment in the semiarid San Juan River. Further work is needed to further elucidate how environmental flows released below dams may be able to assist with maintaining the functional diversity of native fish communities in desert rivers of the American Southwest (Haney et al. 2008; Shafroth et al. 2010).

The functional diversity of non-native communities was prominently explained by regional climate and surrogate measures of hydrologic variation associated with large dams. Our results suggest that watersheds with greater cumulative dam storage capacity and dam density support more functionally diverse communities of non-native fishes, a finding likely reflecting the creation of new niche opportunities for successful establishment (sensu Shea and Chesson 2002). These additional niche opportunities may be associated with both the formation of reservoirs above dams and the modification of downstream flow and thermal regimes. Lentic habitat was historically limited to wetlands and riverine backwaters in the LCRB (Minckley 1973; Carlson and Muth 1989), but today, over 400 large dams and countless small diversions support permanent standing bodies of water (Mueller and Marsh 2002). These novel water bodies provide habitat for many non-native species and are hypothesized to provide superior niche opportunities for the establishment and spread of invasive species (Havel et al. 2005; Johnson et al. 2008). In the LCRB, watersheds with relatively low numbers of dams contain non-native fishes with a narrower range of trait types, more closely resembling the functional breadth of native communities. By contrast, watersheds with higher dam densities support more functionally diverse non-native fish communities, accruing species with equilibrium-type traits that are favored in more stable long-term flow regimes that exist both above and below dams (Olden et al. 2006). Not surprisingly, equilibrium strategists were previously rare in this region given the historically variable hydrologic regime (Olden and Poff 2005). In summary, our results indicate that hydrologic alteration is not only creating niche opportunities expanding the total functional diversity of non-native fish communities, but that those niches are being primarily filled by non-native equilibrium strategist species that are continuing to expand their ranges within the LCRB.

Non-native species also tended to associate with human-altered watersheds characterized by high canal density, roads, agriculture, and urban development. Canal habitat is utilized by a number of non-native fishes for seasonal refuge during low flow periods and can promote the secondary spread of species by increasing landscape connectivity (May and Brown 2002; Cowley et al. 2007). Road construction and urban development have also been identified as having both short- and long-term effects on the physical and chemical conditions in aquatic ecosystems (Angermeier et al. 2004; Wheeler et al. 2005). The ability of many non-native fish species to persist in these altered environments to a greater extent than native species may account for the association of non-native species in those areas. However, many of the land use variables did not significantly influence the functional diversity in our study, suggesting that hydrologic alteration may play a more critical role in shaping the fish communities.

A striking result of our study was that natural and human-related factors explained substantially more variation in functional trait composition compared with taxonomic composition of fish communities. This disparity is expected when species distributions are geographically more limited than the spatial scale of investigation. When multiple species have similar traits but have been historically separated resulting in unique distribution patterns, environmental variation may explain a greater percentage of functional composition variation than taxonomic composition. Our results complement the findings of Hoeinghaus et al. (2007), who found environmental factors explained greater variation in fish functional groups compared with taxonomic composition for major river basins of Texas, USA. Furthermore, although at the watershed level we found taxonomic and functional richness to be positively associated, we expect that functional metrics will more directly capture the mechanistic influence of environmental factors shaping fish community composition. Using species traits as a currency in future community ecology analysis will provide predictability and generality to results and should contribute to general ecological rules associated with competition, predation, and invasion (McGill et al. 2006; Olden et al. 2010).

In conclusion, the landscape factors governing the occurrence of native and non-native species within aquatic environments are inherently complex. A mosaic of anthropogenic landscape drivers interacting with the existing habitat variability appears to dictate the functional composition of fish communities, ultimately defining the species within rivers. With limited resources to invest in the conservation of native fauna and multiple threats simultaneously impacting LCRB biotas (Cowley 2006), effective management of imperiled species depends on determining the primary influences on fishes at a landscape scale. Growing anthropogenic pressures associated with human population growth in the arid Southwest of the United States (Paukert et al. 2010) support the development of multivariate models that provide mechanistic insight into the major drivers of fish community structure. Our results identified multiple landscape-scale environmental influences on fish community composition and diversity; however, hydrologic alteration factors (presence of dams and canals) and seasonally variable precipitation regimes were the most important drivers of fish communities' taxonomic and functional composition. Given that full flow restoration and (or) dam removal in the LCRB is highly unlikely, managing for both native and non-native fish communities may be the most realistic conservation strategy. The implementation of environmental flows to mimic the components of the natural hydrograph is one possible tool to achieve this goal (Valdez et al. 2001; Brown and Ford 2002; Propst et al. 2008). Without acknowledging the critical influence that hydrologic alteration has on fish

community composition, management activities such as mechanical removal of non-native species may have limited long-term success.

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Appendix A

Appendix A appears on the following pages.

Table A1. Variables included in each candidate model describing functional diversity of LCRB watersheds.

		Hydrologic alteration					Land use and physiographic features		
Model	Candidate model	Dam density	Dam storage	Reservoir surface area	Canal habitat	Distance to down dam	Agriculture	Road density	Urban development
1	Global	1	1	1	1	1	1	1	1
2	Hydrologic disturbance	0	1	1	1	1	0	0	0
3	Land use #1	1	0	0	0	0	1	1	1
4	Land use #2	1	0	0	0	0	1	1	1
5	Land use #3	1	0	0	0	0	0	0	0
6	Climate	0	0	0	0	0	0	0	0
7	Climate precip.	0	0	0	0	0	0	0	0
8	Climate precip. CV	1	0	0	0	0	0	0	0
9	Climate temp.	0	0	0	0	0	0	0	0
10	Fragmentation	0	0	0	1	0	0	1	0
11	Natural	0	0	0	0	0	0	0	0
12	Human disturbance #1	0	1	1	1	1	1	1	1
13	Human disturbance #2	1	1	1	1	1	1	1	1
14	Moyle and Light 1996	1	1	0	1	0	1	0	1
15	Gido and Brown 1999 #1	0	1	0	0	0	0	0	0
16	Gido and Brown 1999 #2	0	1	1	0	0	0	0	0
17	Marchetti et al. 2004 #1	0	0	0	1	0	0	0	1
18	Marchetti et al. 2004 #2	1	0	0	1	0	0	0	1
19	Marchetti et al. 2004 #3	0	1	0	1	0	0	0	0
20	Propst et al. 2008	0	0	0	0	0	0	0	0
21	Light and Marchetti 2007	1	1	0	1	0	0	0	1
22	Propst and Gido 2004	0	0	0	0	0	0	0	0
23	Rinne and Miller 2006	1	1	0	0	0	0	0	0
24	Meador et al. 2003	0	0	0	0	0	1	0	1

Note: The number 1 indicates the environmental variable included in the model; 0 indicates the variable was not used in the model. Descriptions of
*Native taxonomic richness was used in the non-native analysis and vice versa.

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				Climate					Diversity
Natural land	Protected land	Canyon habitat	Watershed area	Annual precipitation	Winter precipitation (CV)	Spring precipitation (CV)	Summer precipitation (CV)	Annual temperature	Taxonomic richness*
1	1	1	1	1	1	1	1	1	1
0	0	0	1	0	0	0	0	0	0
1	1	0	1	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0
1	1	0	1	0	0	0	0	0	0
0	0	0	1	1	1	1	1	1	0
0	0	0	1	1	1	1	1	0	0
0	0	0	1	0	1	1	1	0	0
0	0	0	1	0	0	0	0	1	0
0	0	0	1	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	0
0	0	0	1	0	0	0	0	0	0
0	1	0	1	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0	1
0	0	0	1	0	0	0	0	0	1
0	0	0	1	1	0	0	0	0	1
0	0	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	1
0	0	0	1	0	0	0	0	0	1
0	0	1	1	1	1	1	1	0	1
0	0	0	1	0	0	0	0	0	1
0	0	0	1	1	0	1	1	0	1
0	0	1	1	1	1	1	1	0	1
0	0	0	1	1	0	0	0	1	1

variables are in the Materials and methods section.

Management implications of the effects of marine-derived nutrients on salmon population dynamics

Milo D. Adkison

Abstract: Pacific salmon (*Oncorhynchus* spp.) populations transfer large quantities of nutrients from their marine habitats to their freshwater habitats, but the management implications of this nutrient import mechanism are not clear. I investigate whether the effects of these nutrient imports on salmon productivity can be detected, how well management strategies can perform if they ignore the effects of marine-derived nutrients when productivity is strongly dependent on nutrient levels, and under what circumstances nutrient-depleted stocks can recover. I find that stock-recruitment data do not usually permit the detection of the effects of nutrient imports. Some management strategies that ignore nutrient-related effects will, as they are updated, converge to policies that are similar to those that are optimal, given the nutrient effects. Constant harvest rate policies will approach optimal harvesting faster than constant escapement policies. Nutrient dependence can lead to multiple stable states for a population, so that a population subject to one harvest rate can be either maintained at high abundance or, if at low abundance and nutrient-depleted, driven to extirpation. While the importance of nutrient effects on a salmon stock may be difficult to ascertain, managers can employ harvest strategies that are robust to this possibility.

Résumé : Les populations de saumons du Pacifique (*Oncorhynchus* spp.) transfèrent de grandes quantités de nutriments de leurs habitats marins à leurs habitats d'eau douce, mais les conséquences de ce mécanisme d'apport sur la gestion ne sont pas claires. Dans cette étude, il s'agit de savoir s'il est possible de déceler les effets des nutriments sur la productivité des saumons, d'évaluer la performance des stratégies de gestion qui ne tiennent pas compte des effets des nutriments étant donné la forte dépendance des nutriments et de connaître les circonstances dans lesquelles les stocks appauvris en nutriments peuvent se rétablir. Les données de stock-recrutement ne permettent pas généralement de déceler les effets des nutriments. Certaines stratégies de gestion qui négligent les effets des nutriments vont, à mesure qu'elles sont mises à jour, converger vers des politiques qui ressemblent à celles qui sont optimales en ce qui a trait aux effets des nutriments. Les politiques de récolte continue vont atteindre les récoltes optimales plus rapidement que les politiques d'échappement constant. La dépendance des nutriments peut mener à de multiples états stables dans une population, avec la conséquence qu'une population soumise à un taux de récolte donné peut ou bien être maintenue à une abondance élevée, ou alors être poussée à l'extirpation si elle a une abondance faible et est appauvrie en nutriments. Bien que l'importance des effets des nutriments sur un stock de saumons puisse être difficile à déterminer, les gestionnaires peuvent employer des stratégies de récolte qui sont robustes compte tenu de cette possibilité.

[Traduit par la Rédaction]

Introduction

For decades, managers have been aware that salmon carcasses contain substantial amounts of marine-derived nutrients (MDN) such as nitrogen, phosphorus, and energy-rich organic materials, and that these imports can be a significant fraction of the nutrients present in freshwater systems (Juday et al. 1932; Mathisen 1971; Kline et al. 1990). These MDN are used by a variety of components of the ecosystem (Willson and Halupka 1995; Naiman et al. 2002; Reimchen et al. 2003). In recent decades, human activities have resulted in significant reductions in the amount of MDN reaching many watersheds (Larkin and Slaney 1997; Gresh et al. 2000), even in watersheds with healthy salmon populations (Finney et al. 2000; Schindler et al. 2005; Adkison 2010).

Nutrient levels have been augmented either by fertilization with inorganic nutrients (Hyatt and Stockner 1985; Kyle et al. 1997; Slaney et al. 2003) or with raw or processed salmon carcasses (Michael 2003; Compton et al. 2006). Several authors have called for increasing escapement goals to meet ecosystem needs for MDN (Michael 1998; Bilby et al. 2001). However, the motivation for many of these fertilization programs has been to increase the productivity of the salmon stock itself.

Although juvenile salmon have been shown to incorporate MDN in their tissues (Kline 2003; Uchiyama et al. 2008), to feed on salmon carcasses and eggs (Bilby et al. 1998; Hicks et al. 2005), and to exhibit improved growth rates and condition when carcass abundance increases (Bilby et al. 1998; Wipfli et al. 2003; Lang et al. 2006), evidence that increasing MDN increases the productivity (as adult recruits per

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spawner) of salmon stocks is equivocal (Schindler et al. 2005; Uchiyama et al. 2008; Piccolo et al. 2009).

In a previous study (Adkison 2010), I showed that salmon populations heavily dependent on marine-derived nutrients were often better managed more conservatively. Lower harvest rates would maintain high escapements and high levels of nutrients, resulting in higher productivity and higher sustained catches. Several practical questions remained unaddressed, such as "how do we identify which stocks are dependent on marine-derived nutrients?" and "what are the consequences of ignoring nutrient effects when they exist?"

In this study, I try to provide practical guidance for the managers of salmon stocks. I examine (*i*) our ability to detect the effects of marine-derived nutrients on the productivity of salmon stocks using spawner and recruitment data, (*ii*) the performance of management strategies that ignore nutrient effects when applied to salmon stocks that are heavily nutrient-dependent, and (*iii*) the potential for recovery of nutrient-dependent stocks once population abundance is decreased and nutrient levels are depleted.

Materials and methods

The model

I used a one-stage Beverton–Holt model (Hilborn and Walters 1992, their Table 7.2) of the relationship between the abundance of spawners and the number of adult offspring that resulted.

$$R_{t+1} = \frac{aS_t}{1 + \frac{a}{b}S_t} \quad (1)$$

$$S_t = R_t - C_t \quad (2)$$

Here, S indicates spawning adults, R is recruits (adult offspring), C is fisheries harvest, and the subscript t denotes generation. For simplicity, I used a generation time of one year; although this is not typical of Pacific salmon, the assumption simply accelerates trends relative to more realistic life histories but does not affect the qualitative conclusions of the analyses. The parameter a is the number of recruits per spawner expected at low spawner densities, whereas b is the upper limit on average recruitment (the average recruitment at very high densities of spawners). The parameter b scales the size of the population, but does not affect its dynamic behavior; to facilitate display of results, small values of b were generally used. This model can be modified to incorporate nutrient effects by making one of the parameters (either a or b) a function of nutrient levels as described below.

The nutrient pool

Import of marine-derived nutrients was assumed to be proportional to the number of spawning salmon. A fraction of the nutrients ($1-\lambda$) were assumed to be lost every year to export. The pool of nutrients at any one time was thus modeled as proportional to a weighted average of prior escapements, i.e., measured in units of carcasses (Uchiyama et al. 2008), as follows:

$$N_t = \sum_{j=0}^{\infty} S_{t-j}\lambda^j \quad (3)$$

Nutrient effects

A parameter of the model — either an a or b — was modeled as dependent upon the nutrient pool derived from all previous generations of spawners (Adkison 2010):

$$p_t = [p_{min} + (p_{max} - p_{min})] \times \left(1 - e^{\frac{\ln(2)N_t}{\gamma}}\right) \quad (4)$$

The parameter increases with nutrient concentration (N) to an asymptotic limit; the minimum value of the parameter is p_{min}, the maximum value is p_{max}, and when $N = \gamma$, the parameter value would be the midpoint of the two extremes.

Probability of detecting of nutrient effects using stock-recruit data

Two hypothetical populations were examined, in which either the parameter a or b was strongly affected by nutrient levels. Parameter values for both of these populations (Table 1) were chosen so that the decline in productivity resulting from overexploitation was severe relative to that of a similar population where parameter values were set at a_{max} and b_{max} (Table 1) and were unaffected by nutrient levels.

I looked at a range of possible historical management and data scenarios to determine under what circumstances the effect of nutrients might be evident in stock and recruitment data. Specifically, I examined the effect of the number of years of spawner and recruitment observations available, the magnitude of the natural variability about the spawner–recruitment relationship, and the effect of strong contrast in the time series of spawning abundance and nutrient levels. The number of years of data ranged from 10 to 50, and natural variability in the logarithm of recruitment ranged from 0.1 to 0.9.

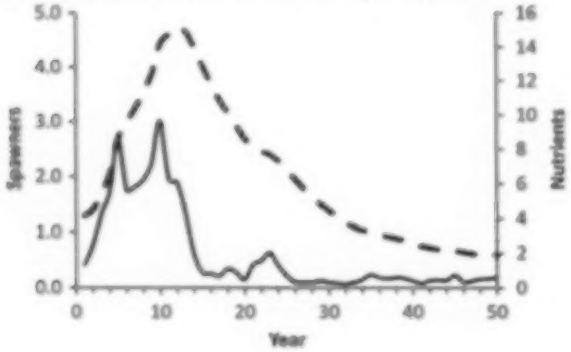
For the highest contrast scenario, the harvest rate was set at 0.20 for the first 10 years, then 0.75 for the rest of the simulation; the true optimal harvest rate was intermediate between these two extremes (Table 1). The abundance in the first year of the simulation was set at some fraction of the unfished abundance (for calculating initial nutrient levels this abundance was assumed to also apply to previous years). The initial abundance fractions explored ranged from 0.10 to 0.90. The effect of low initial abundances and low initial harvest rates was to produce an increasing abundance for the first 10 years of the simulation, followed by a decrease when an excessive harvest rate was applied beginning in year 11 (Fig. 1).

To assess whether nutrient effects would be detected from the simulated time series, I fit two competing models to each set of simulated data. The first model was the Beverton–Holt model given by eq. 1, with the maximum likelihood values of the parameters a and b estimated using a non-linear search algorithm. The second model was the same, except that a parameter (either a or b) was correctly modeled as being affected by nutrients according to the relationship specified by eq. 4, so that four parameters (e.g., a, b_{min}, b_{max}, and γ) were estimated. In the model incorporating nutrient effects, the parameter λ was assumed known (given simple assumptions about nutrient fates, calculation may be possible based on physical characteristics of the watershed such as the flushing rate; Uchiyama et al. 2008). The fits of the two models were compared using the sample-size-cor-

Table 1. Parameter values and management characteristics of simulated populations in tests of the detectability of nutrient effects.

Parameter	*a*-limited population	*b*-limited population
a_{min}	3	6
a_{max}	9	6
b_{min}	5	1
b_{max}	5	5
γ	4	4
λ	0.9	0.9
$S_{unfished}$	4.44	4.16
S_{opt}	1.28	1.43
h_{opt}	0.624	0.525

Fig. 1. One realization of a simulated high contrast time series, starting from 10% of the unfished abundance. The stock is underexploited for the first 10 years, then overexploited for the remainder. In this example, the parameter *a* is strongly dependent on nutrient levels (in units of carcasses). The number of spawners is given by the solid line, the nutrient level by the broken line.



rected Akaike's information criterion (AICc; Burnham and Anderson 1998), correctly assuming normal error in the logarithm of recruitment.

The nutrient influence was scored as detected when the AICc score of the model incorporating the nutrient effect was lower than that of the model without the nutrient effect. I ran 1000 trials and recorded the fraction of simulations in which the nutrient effect was detected.

Yield from naive management

I simulated a salmon stock that was heavily dependent on marine-derived nutrients. I compared the performance (yield and escapement levels) of management strategies that might be employed by managers unaware of the dependence of the stock on marine-derived nutrients to strategies that were optimal given the nutrient dependence. Optimal strategies were found by simulation, searching over a wide range of harvest rates or escapement goals for those that maximized long-term yield.

The population dynamics model used in simulations was the single-stage stock-recruitment model (eqs. 1–2), with a log-normal error in recruitment (coefficient of variation, CV = 0.3) and an asymptotic relationship (eq. 4) between nutrients and one of the parameters (either *a* or *b*). Whatever the management strategy, the simulation included management error (Holt and Peterman 2006); i.e., log-normal error in the escapement actually achieved (CV = 0.1).

The simulation began with managers either using the optimal escapement goal or one that was 50% below or above optimal. This goal was applied for the first 10 years of the simulation. The simulation ran an additional 25 years, with managers updating their stock-recruitment fit using maximum likelihood at the 10th year and every 5 years thereafter. Managers then changed their management goal to that which would be optimal given their current estimates of the stock-recruitment relationship. Management strategies evaluated were constant escapement strategies and constant harvest rate strategies. For additional realism, managers were unable to change a management target by more than 20% at each update.

I tracked the evolution of the estimates of the optimal harvest strategy, as either the harvest rate or escapement goal. I also tracked escapements and yields, and compared them with those that would have resulted had the optimal strategy for the nutrient-affected stock been applied beginning in the 11th year.

Recovery of a nutrient-depleted stock

I calculated the deterministic population trajectory of a salmon stock that was heavily dependent on marine-derived nutrients, starting from a range of initial population abundances (1% to 100% of the unfished level) and subject to a range of harvest rates (1% to 99%). The initial nutrient level was assumed to be in equilibrium with the initial abundance; i.e., $N_0 = S_0/(1-\lambda)$.

Results

Detectability of nutrient effects using stock-recruit data

In simulations, even large effects of nutrients on a parameter of the stock-recruitment relationship had a low probability of being detected, with the exception of a few unrealistic scenarios; that is, when the CV of the natural logarithm of escapement was only 0.1 and the simulations started with an abundance of 1/10 the unfished equilibrium (top left of both parts of Table 2, in bold). When larger, more typical CVs were used in the simulations, the probability of detection was low, even with many years of data and high contrast in stock sizes and nutrient levels. Likewise, when initial abundances were higher so that the increase in abundance was not so pronounced in the first 10 years of the simulation, the probability of detecting the influence of nutrients was small.

Yield from naive management

Naive management approaches, where managers set escapement goals or harvest rates without accounting for the potential effects of nutrients on stock productivity, evolved towards near optimal policies in all trials (Fig. 2). However, the rate at which they evolved varied widely among scenarios. In scenarios where there was an initial 10 years of managing for the true optimal escapement or harvest rate, new estimates of optimal escapement or harvest rate from naive models tended to maintain the current strategy. When the initial 10 years were from a suboptimal strategy, managing using a constant harvest rate (Figs. 2*c* and 2*d*) produced faster

Table 2. Probability of detection (from 1000 simulations) of nutrient effects in either the parameter b or parameter a as a function of the initial escapement (as initial S/unfished S) and the coefficient of variation (CV) in the natural logarithm of recruitment.

	Initial S/unfished S				
CV	0.1	0.3	0.5	0.7	0.9
b					
0.1	**0.91**	0.26	0.20	0.19	0.19
0.3	0.19	0.06	0.07	0.08	0.08
0.5	0.07	0.02	0.04	0.05	0.05
0.7	0.04	0.02	0.02	0.02	0.03
0.9	0.02	0.01	0.01	0.01	0.02
a					
0.1	**0.53**	0.05	0.04	0.04	0.04
0.3	0.13	0.01	0.00	0.00	0.00
0.5	0.07	0.00	0.00	0.00	0.00
0.7	0.04	0.00	0.00	0.00	0.00
0.9	0.02	0.00	0.00	0.00	0.00

Note: Values > 0.5 included in bold.

evolution towards the optimal strategy than managing using an escapement goal (Figs. 2*a* and 2*b*). This faster learning was probably due to the higher contrast in escapements that resulted from using a constant harvest rate compared with that when the escapement goal was constant. With an escapement goal strategy, faster convergence towards the optimal strategy was seen when the stock was underexploited compared with when it was initially overexploited (Figs. 2*a* and 2*b*).

Recovery of a nutrient-depleted stock

For unproductive stocks where the parameter a was heavily dependent on nutrients, two stable states were possible. If the initial abundance and nutrient levels were low, the stock could be driven to extirpation by moderate harvest pressure. If the stock initially was unfished, the same harvest rates would maintain the stock at high abundance. For example, the parameter combination $a_{min} = 1$, $a_{max} = 5$, $b = 6$, $\gamma = 4$, and $\lambda = 0.9$, results in a population whose fate (Fig. 3*a*) and maximum sustainable harvest pressure (Fig. 3*b*) is heavily dependent on the initial population abundance and nutrient levels.

This dependence decreases as the minimum per capita productivity (a_{min}) increases. In the example shown (Fig. 3), if the value of a_{min} is increased to 3, there is little effect from the initial abundance (not shown). Similarly, if the parameter value increases more rapidly with nutrient levels, initial abundance is less important. For example, if γ is decreased to 0.5, only very small populations (1% or less) differ in behavior from large populations, and then only for a very narrow range of harvest rates (not shown). Multiple stable states were not seen when the parameter b depended on nutrient levels.

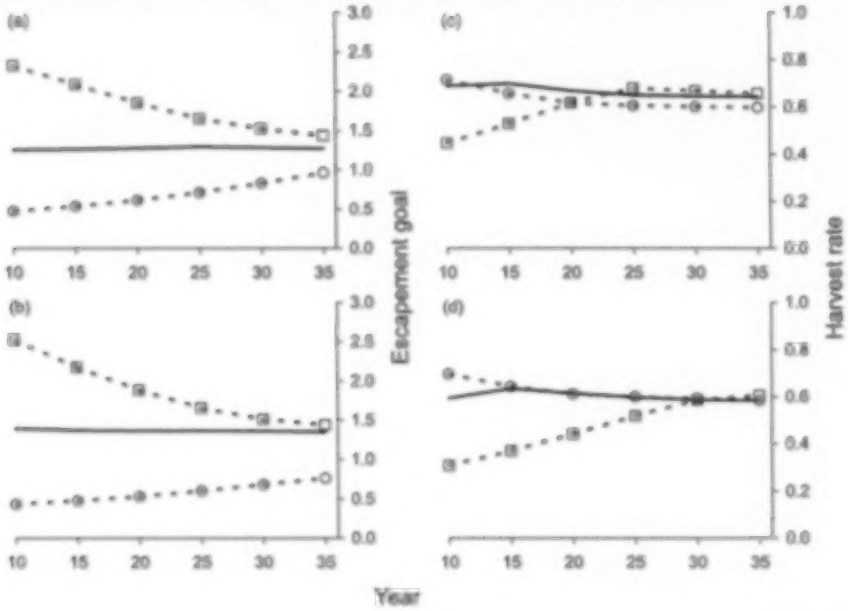
Discussion

Even when the productivity of a salmon population is highly dependent on MDN in the form of adult carcasses, this phenomenon is unlikely to be detected in the spawner–recruit data. The simulated scenario investigated here was more favorable for detecting the effects of marine-derived nutrients than would be likely with real world data. Parameters were chosen so that the nutrients had a strong influence on stock productivity. The simulated data available for analysis were a long time series containing large contrasts in both escapement levels and in nutrient concentrations. Yet, only at unrealistically low levels of environmental stochasticity (CV = 0.1) was there any substantial probability of detecting the influence of the nutrients. Uchiyama et al. (2008) were unable to find any effect of nutrients in the spawner–recruitment data for 16 sockeye salmon (*Oncorhynchus nerka*) stocks, despite a strong relationship between the number of spawners and MDN levels in smolts from many of these stocks. However, this analysis shows that these tests probably had little statistical power, and strong effects of nutrients might well have been missed.

The basic problem with trying to detect nutrient effects on the relationship between spawners and recruitment is that these effects will be strongest for the offspring of the spawners, with the MDN derived from any group of spawners having diminishing effects on later cohorts (eq. 3). Unfortunately, a relationship between spawners and their offspring is central to the spawner–recruit models, often generalized as recruits = f(spawners), that constitute the null hypothesis of no nutrient effects (e.g., eq. 1), and only the weaker, persistent effects on later cohorts are available to provide any statistical power. In summary, a test of nutrient effects on stock productivity using stock and recruitment data is effectively based on weak interactions among cohorts, and is unlikely to detect even strong effects of nutrients on productivity. Studies focusing on ecosystem processes (e.g., Hicks et al. 2005), direct effects on fish health and survival (e.g., Bilby et al. 1998; Heintz et al. 2004), retrospective analyses (e.g., Finney et al. 2000; Schindler et al. 2005), and using replicates and controls (e.g., Wipfli et al. 2003; Lang et al. 2006; Kohler et al. 2008), are more promising ways to demonstrate an effect.

Some previous modeling efforts have suggested that optimal harvest policies may be affected by strong nutrient effects (Knudsen et al. 2003; Hamazaki 2009). In a previous

Fig. 2. The evolution of naive management strategies, either an escapement goal (as adult spawners; panels *a* and *b*) or harvest rate (the fraction harvested; panels *c* and *d*) over time. In the top graphs (*a* and *c*), the parameter *a* is dependent on nutrients; in the bottom graphs, parameter *b*. The initial strategy is either optimal for the stock (solid line), overexploits it (broken line with circles), or underexploits it (broken line with squares). The management strategy is re-evaluated every 5 years, beginning in year 10.



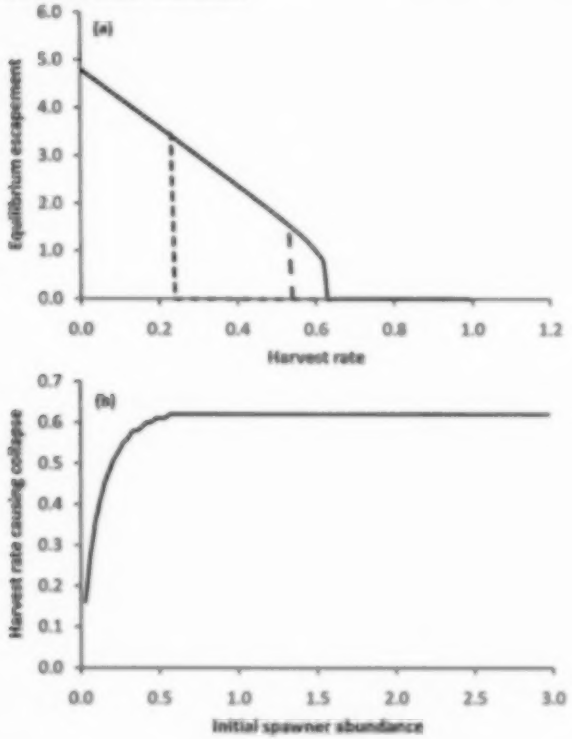
article (Adkison 2010), I showed that maintaining high escapement levels could result in higher catches over the long term, if the nutrients derived from the salmon carcasses were necessary to maintain high productivity of the salmon stock. However, I also found some circumstances in which higher escapements did not result in higher catches; for instance, the additional escapement required to maintain high productivity could be larger than the increase in the sustainable catch. Another important exception was where the salmon population was limited by multiple factors, some of which were not ameliorated by increased nutrient levels (e.g., a limited area for spawning).

Fortunately, the simulations presented here suggest that naive management strategies may perform adequately even when strong nutrient effects exist. Bernard and Clark (2009), using a quite different model of nutrient effects, similarly found that conventional management approaches worked well. In my simulations, naive management strategies tended to evolve towards escapement goals or harvest rates that were near optimal for the nutrient-dependent population. This evolution happened most rapidly when the management strategy provided contrast (e.g., a large range) in the abundance of spawners, and when the stock was at high abundance. The recovery could be quite slow for a stock that was initially overharvested and then managed using an escapement goal policy.

The modeling presented here also suggests that overharvested nutrient-dependent stocks can be driven into a nutrient deficiency trap, where the population is unable to sustain the harvest rates it could support, were its abundance higher. Similar mechanisms have been proposed to explain the collapse and lack of recovery of many salmon stocks (Schmidt et al. 1998; Gresh et al. 2000). To effect recovery of depleted (or to increase healthy) stocks, fertilization with either chemicals or salmon carcasses has been used (Stockner and McIsaac 1996; Michael 2003; Slaney et al. 2003), although potential drawbacks, such as excessive nutrients, toxins, and disease, have been listed (Compton et al. 2006).

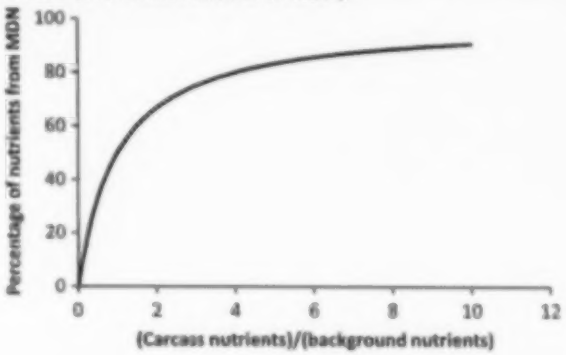
One likely misguided strategy for determining the amount of carcasses or nutrients for supplementation has been based on results by Bilby et al. (2001), who found that the fraction of MDN in juvenile coho salmon tissues did not increase appreciably when the mass of adult carcasses exceeded 0.15 kg·m^{-2}. This has been interpreted as a "saturation" level of carcass-derived nutrients, beyond which juvenile salmon receive little benefits (Bilby et al. 2001), and has used to set target levels of carcass additions to watersheds in both Washington and Oregon (Michael 2005; Shaff and Compton 2009). However, this phenomenon of MDN "saturation" is partly driven by mathematics, and may not have any ecological implications. For example, assuming MDN was spread throughout the ecosystem in proportion to its concentration, the import of carcasses sufficient to quadruple the background level of nutrients would result in 75% MDN in tissues (ignoring qualitatively unimportant physiological and ecological complications in the incorporation of MDN into tissues) (Fig. 4). However, carcass levels resulting in an eightfold increase over background would result in only a slightly higher concentration, 87.5% (Fig. 4). More importantly, this same minor increase in MDN in tissues would happen irrespective of whether the population was food-limited at either the basal nutrient level or at either level of carcass additions.

Fig. 3. Multiple stable states when the parameter a is heavily dependent on nutrient levels ($a_{min} = 1$, $a_{max} = 5$, $b = 6$, $\gamma = 4$, $\lambda = 0.9$). Panel (a) shows the equilibrium spawning escapement (number of spawning adults) as a function of harvest rate (fraction harvested) for populations starting at either 100% (solid line), 5% (long dashes), or 1% (short dashes) of the unfished abundance; a population starting at higher abundance can sustain a higher harvest rate without collapsing. Panel (b) shows the maximum harvest rate a population can sustain without collapsing as a function of its initial abundance. In both panels, initial nutrient levels are in equilibrium with initial abundance.



The fraction of MDN incorporated into juvenile salmon tissues is not necessarily indicative of the satisfaction of the demand for these nutrients. In fact, high levels are likely to indicate a high demand, and low levels the opposite. For example, in ecosystems rich in nutrients from non-marine sources, food for juvenile salmon might be sufficient to their needs even without MDN. In such systems, carcasses could constitute a small fraction of the total nutrients in the ecosystem. The resultant low fraction of MDN in juvenile salmon tissues would be indicative of a low demand rather than an indication that more carcasses are needed. Conversely, in nutrient-poor systems we should expect a high fraction of MDN in juvenile salmon tissues, even when the total food ration remains quite low relative to the population's needs. If the juvenile salmon directly consumed carcasses or eggs, the fraction of MDN in tissues could approach 100%, at least seasonally (Reichert et al. 2008), but again this would happen irrespective of whether the food supply was sufficient.

Fig. 4. The percentage of marine-derived nutrients (MDN) in the ecosystem as a function of the quantity of MDN (in units of adult carcasses) delivered to the system relative to the background nutrient level (i.e., nutrients not due to MDN).



The current management strategy of using a high percentage of MDN in juvenile salmon as an indication they are receiving sufficient MDN does not hold up under close inspection. Levels of carcass and nutrient additions should be based on more direct measures of the population's response, such as the number of smolts produced, or their condition. There have been several studies of the response of freshwater ecosystems to carcass abundance (e.g., Bilby et al. 2001; Wipfli et al. 2003; Shaff and Compton 2009), artificial carcass analogs (Kohler et al. 2008), and chemical fertilization (e.g., Stockner and McIsaac 1996; Bradford et al. 2000; Slaney et al. 2003). Based on this literature, recommendations for target levels and effective dosing strategies have been made (Ashley and Stockner 2003; Michael 2005). Reviews of this literature (Ashley and Stockner 2003; Compton et al. 2006), including meta-analytic techniques (Janetski et al. 2009), should provide some generalized guidelines that could be customized to the particulars of an individual watershed.

In this study, I have simulated salmon populations highly dependent on marine-derived nutrients to maintain their productivity. The intent was to provide the managers of such stocks, or of stocks where such dependence cannot be ruled out, with practical guidance for setting harvest levels. Fortunately, the guidance for management is straightforward, and the recommended actions will probably also suit stocks that are not dependent on marine-derived nutrients. I found that naive management strategies could be successful, particularly when they incorporate the adaptive management (Walters 1986) principles of periodic re-evaluation and ensuring contrast in stock sizes. Specifically, periodically re-evaluating a constant harvest rate strategy ensured variation in stock size and fairly rapidly converged to harvest rates that were nearly optimal for nutrient dependent stocks. This policy could be adopted as a robust strategy when the degree of dependence on marine-derived nutrients is unknown.

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Effects of copper, cadmium, lead, and arsenic in a live diet on juvenile fish growth

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Abstract: The effects of diet-borne copper, cadmium, lead, and arsenic on juvenile fish were evaluated using a live diet consisting of the oligochaete *Lumbriculus variegatus*. In 30 d exposures, no effects were observed on the growth and survival of rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), and channel catfish (*Ictalurus punctatus*) fed diets contaminated with copper [130–310 μg Cu·(g dm)$^{-1}$], cadmium [90–540 μg Cd·(g dm)$^{-1}$], and lead [850–1000 μg Pb·(g dm)$^{-1}$]. However, rainbow trout growth was reduced in a dose-dependent manner for diets contaminated with arsenic [26–77 μg As·(g dm)$^{-1}$]. These effects of arsenic on fish growth were accompanied by slower feeding rate, reduced food conversion efficiency, liver cell abnormalities, and fecal matter changes suggestive of digestive effects, and occurred to a similar extent whether the diet was exposed to arsenate or arsenite. Effects from these dietary levels of arsenic, and the absence of effects from these dietary levels of metals, were generally consistent with literature reports using laboratory diets amended with toxicant salts. These results also indicated that reported growth effects on rainbow trout fed diets of invertebrates collected from mining-contaminated areas of the Clark Fork River (Montana, USA) or exposed in the laboratory to Clark Fork River sediments are likely more attributable to the arsenic than the metals in those diets.

Résumé : Nous avons évalué les effets du cuivre, du cadmium, du plomb et de l'arsenic dans le régime alimentaire sur de jeunes poissons en utilisant des proies vivantes, des oligochètes *Lumbriculus variegatus*. Au cours d'expositions de 30 j, nous n'avons observé aucun effet sur la croissance et la survie de truites arc-en-ciel (*Oncorhynchus mykiss*), de têtes-de-boule (*Pimephales promelas*) et de barbues de rivière (*Ictalurus punctatus*) de régimes alimentaires contaminés par le cuivre [130–310 μg Cu·(g masse sèche)$^{-1}$], le cadmium [90–540 μg Cd·(g masse sèche)$^{-1}$] et le plomb [850–1000 μg Pb·(g masse sèche)$^{-1}$]. Cependant, la croissance de la truite arc-en-ciel est réduite en proportion directe de la dose par un régime contaminé par l'arsenic [26–77 μg As·(g masse sèche)$^{-1}$]. Les effets de l'arsenic comprennent une alimentation ralentie, une efficacité réduite de la conversion de nourriture, des anomalies dans les hépatocytes et des changements dans les fèces, ce qui semble représenter un impact sur la digestion; ces effets se produisent à la même intensité que le régime soit exposé à l'arséniate ou à l'arsénite. Les effets produits par ces concentrations alimentaires d'arsenic et l'absence d'effets des concentrations alimentaires de métaux s'accordent assez bien avec les résultats de la littérature basés sur des régimes alimentaires modifiés au moyen de sels de substances toxiques. Ces résultats indiquent aussi que les effets signalés chez les truites arc-en-ciel nourries de régimes d'invertébrés récoltés dans des zones contaminées par les opérations minières dans la rivière Clark Fork (Montana, É.-U.) ou exposées en laboratoire à des sédiments provenant de la Clark Fork sont plus probablement attribuables à l'arsenic qu'aux métaux dans le régime.

Introduction

Assessments of risks to aquatic animals from metals and metalloids generally characterize effects based on laboratory toxicity tests in which the toxicant is added to the exposure water, but not food, so that exposure via diet relative to water is likely less than in contaminated ecosystems. However, various laboratory studies have demonstrated that metal/metalloids in food can cause toxicity and might be an important component of risk for some toxicants, organisms, and exposure conditions (Meyer et al. 2005). This is of particular concern for sites where metal/metalloid exposures arise primarily from contaminated sediments, so that exposure of water-column organisms via sediment-based food chains would have increased importance relative to exposure from water. Woodward et al. (1994, 1995) reported reduced growth of juvenile rainbow trout (*Oncorhynchus mykiss*) fed diets prepared from benthic macroinvertebrates collected from sites on the Clark Fork River, Montana, USA (CFR) with highly contaminated sediments, compared with fish fed diets derived from less contaminated portions of the CFR or other sources.

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Using field-collected diets addresses concerns that toxicity studies using salt-amended, artificial diets have uncertain relevance to effects of contaminated food in natural systems, because of possible differences in the chemical speciation of the contaminants within the diets (Suedkamp 1999; Clearwater et al. 2002). However, field-collected diets can also create difficulties, such as diet quality varying among the sites, thereby confounding contaminant effects. Such compositional differences, especially for lipids, create uncertainties regarding the effects reported by Woodward et al. (1994, 1995) and the application of these effects to risk assessments.

Application of the findings of Woodward et al. (1994, 1995) to risk assessments is also made difficult by their use of diets containing a mixture of toxicants with uncertain relative effects. Of the various metals measured in the CFR diets, copper was highest relative to diet concentrations reported to be toxic to salmonid fish in laboratory studies using diets amended with metal salts, but CFR dietary copper concentrations [170–415 μg Cu·(g dry mass)$^{-1}$, (dm)] were still substantially below the concentrations found to be toxic in these laboratory studies (>500 μg Cu·(g dm)$^{-1}$; see Clearwater et al. 2002; Borgmann et al. 2005). In contrast, arsenic concentrations in the CFR diets showing growth effects [19–43 μg As·(g dm)$^{-1}$] were near or in the range reported to reduce rainbow trout growth [33–49 μg As·(g dm)$^{-1}$] based on laboratory studies with arsenate-amended diets (Cockell and Hilton 1988; Cockell et al. 1991), making this contaminant of more likely concern.

The inherent difficulties in interpreting and applying these data indicated a need for laboratory studies designed to better address nutritional issues and the effects of the individual contaminants, while still using diets that are more representative of those in natural systems. Mount and co-workers (Mount et al. 2006) proposed the use of the oligochaete worm *Lumbriculus variegatus* for such purposes, and demonstrated its efficacy as a fish diet. This organism can be exposed over an extended duration to allow more realistic speciation and distribution of contaminant within the diet, and has the advantage of being able to be exposed to contaminated sediment and (or) water. Using this approach, Hansen et al. (2004) evaluated the survival and growth of rainbow trout fed diets of *L. variegatus* that had been exposed to sediments collected from two contaminated sites in the CFR watershed and from an uncontaminated reference stream, and found reduced growth for rainbow trout fed *L. variegatus* that had been exposed to the more contaminated sediments, consistent with the findings of Woodward et al. (1994, 1995).

However, the causative agents of this reduced growth are still unresolved, and this information has uncertain applicability to other fish species, sites, and contaminants. Information is therefore needed regarding the effects of individual contaminants, the sensitivities of different fish species, and the roles of various site characteristics, including chemical speciation, diet composition, routes of exposure, and various physicochemical factors. As part of a larger effort to provide such information, the efforts described in this paper evaluated the effects of diets consisting of *L. variegatus* contaminated separately with copper, cadmium, lead, and arsenic, on the growth and survival of multiple fish species.

Materials and methods

Study design

Six experiments were conducted to test the effects of diet-borne exposures for various metal/metalloids, dietary toxicant concentrations, and fish species (Table 1). All experiments were 30 d in duration. Experiments 1–4 consisted of 4 replicates of 3 or 6 dietary treatments (including the control), with growth measured only at test termination. Experiments 5 and 6 consisted of eight replicates of three dietary treatments, with growth measured in half of the replicates at 15 d and the rest at test termination.

In Experiment 1, the effects of dietary cadmium and lead on rainbow trout were determined at a single level of each metal in the diet, obtained by exposing the *L. variegatus* to sediment spiked with the metals. Because this, and some unreported preliminary experiments, revealed difficulties in achieving consistent and high dietary metal levels by such sediment exposures, subsequent experiments used *L. variegatus* exposed via water. Experiments 2 and 3 exposed channel catfish (*Ictalurus punctatus*) and fathead minnow (*Pimephales promelas*) to two levels of cadmium, two levels of copper, and one level of lead in the diet. Experiment 4 exposed rainbow trout to two levels of dietary copper. Experiments 5 and 6 exposed rainbow trout to dietary arsenic using worms exposed to two levels of arsenate (Experiment 5) or two levels of arsenite (Experiment 6). The levels of exposure to *L. variegatus* were selected based on preliminary tests to determine exposure concentrations that were not overtly toxic to *L. variegatus* but provided dietary exposures to the fish comparable to or higher than those occurring in CFR-derived diets associated with growth effects.

Test water and organisms

Portions of this work that were conducted at the US Environmental Protection Agency Mid-Continent Ecology Division (MED) (Duluth, Minnesota) used water from Lake Superior that were obtained directly from the lake, and subsequently filtered, UV-treated, and temperature conditioned. This water has a typical pH of 7.5–8.0, a hardness of 45–50 mg $CaCO_3 \cdot L^{-1}$, and an alkalinity of 40-45 mg $CaCO_3 L^{-1}$. Portions of this work that were conducted at Sobran, Inc. (Duluth) used dechlorinated Duluth city water, also of Lake Superior origin. For Experiments 1 and 2, rainbow trout and channel catfish were obtained as late sac-fry from Aquatic Research Organisms (Hampton, New Hampshire). Fish were maintained in flow-through Lake Superior water (11 °C for rainbow trout, 25 °C for channel catfish) and fed starter trout chow ad libitum until test start (13 d after start-of-feeding for rainbow trout, 15 d for channel catfish).

For Experiment 3, fathead minnows were from the in-house culture (MED), which is maintained in Lake Superior water at 25 °C. A cohort of newly hatched fry (0–24 h old) from multiple spawnings was isolated and fed brine shrimp nauplii ad libitum until the test started (30 d after start of feeding).

For Experiments 4–6, rainbow trout were obtained as eggs or sac-fry from the US Geological Survey Upper Midwest

Table 1. Experimental treatments.

Initial fish wet mass ($g \cdot fish^{-1}$)[a]	*Lumbriculus variegatus* exposure route	Treatment	*Lumbriculus variegatus* exposure concentration	Duration (d)
Experiment 1. Rainbow trout				
0.116 (0.008)	Sediment	Control		30
		Cd	3.0 mg Cd·(g dm)$^{-1}$	30
		Pb	6.9 mg Pb·(g dm)$^{-1}$	30
Experiment 2. Channel catfish				
0.213 (0.015)	Water	Control		30
		Low Cd	89 μg Cd·L^{-1}	30
		High Cd	147 μg Cd·L^{-1}	30
		Low Cu	102 μg Cu·L^{-1}	30
		High Cu	151 μg Cu·L^{-1}	30
		Pb	576 μg Pb·L^{-1}	30
Experiment 3. Fathead minnow				
0.114 (0.017)	Water	Control		30
		Low Cd	95 μg Cd·L^{-1}	30
		High Cd	144 μg Cd·L^{-1}	30
		Low Cu	102 μg Cu·L^{-1}	30
		High Cu	150 μg Cu·L^{-1}	30
		Pb	628 μg Pb·L^{-1}	30
Experiment 4. Rainbow trout				
0.413 (0.023)	Water	Control		30
		Low Cu	35 μg Cu·L^{-1}	30
		High Cu	52 μg Cu·L^{-1}	30
Experiment 5. Rainbow trout[b]				
0.151 (0.013)	Water	Control		15, 30
		Low arsenate	3.9 mg As·L^{-1}	15, 30
		High arsenate	7.9 mg As·L^{-1}	15, 30
Experiment 6. Rainbow trout[c]				
0.242 (0.017)	Water	Control		15, 30
		Low arsenite	1.5 mg As·L^{-1}	15, 30
		High arsenite	3.2 mg As·L^{-1}	15, 30

Note: dm, dry mass. For control treatments, water concentrations were <3 μg Cu·L^{-1}, <3 μg Cd·L^{-1}, <12 μg Pb·L^{-1}, and <0.2 mg As·L^{-1}.

[a]Estimated average (standard error) initial wet mass for $n = 8$ fish placed in each experimental chamber, based on wet masses of a separate sample of 20–30 fish from initial pool of fish.

[b]As, arsenate ($Na_2HAsO_4 \cdot 7H_2O$).

[c]As, arsenite ($NaAsO_2$).

Environmental Sciences Center (LaCrosse, Wisconsin). Fish were maintained in flow-through Lake Superior water at 11 °C. Upon swim-up, fish were fed starter trout chow ad libitum until the test started (11–20 d after start of feeding).

Except for Experiment 3, fish were also provided with uncontaminated *L. variegatus* prior to the start of exposure to introduce them to this source of food. Even without this prior introduction, the fathead minnows of Experiment 3 accepted the diet change quite readily, consuming all the offered ration of worms on the first day of exposure. *L. variegatus* were obtained from cultures maintained at MED or at Sobran, cleaned of culture substrate, and exposed to test chemical as described below.

Test chemicals

All test chemicals used for contaminating diets were reagent grade. Lead nitrate ($Pb(NO_3)_2$; CAS No. 10099-74-8) was obtained from J.T. Baker Chemical Company (Phillipsburg, New Jersey). Cadmium chloride ($CdCl_2$; CAS No. 10108-64-2) was from Aldrich Chemical Company (Milwaukee, Wisconsin). Copper sulfate ($CuSO_4 \cdot 5H_2O$; CAS No. 80867-41-0) was obtained from Sigma Chemical Company (St. Louis, Missouri). Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$; CAS No. 10048-95-0) and sodium arsenite ($NaAsO_2$; CAS No. 7784-46-5) were from Sigma-Aldrich (St. Louis, Missouri).

Diet contamination

Lumbriculus variegatus exposures were conducted at Sobran for Experiments 1–3 and at MED for Experiments 4–6. For Experiment 1, contaminated sediments for the worm exposures were created by adding cadmium chloride [30 mmol·(g dm)$^{-1}$] or lead nitrate [40 mmol ·(g dm)$^{-1}$] to sieved (No. 50 mesh) West Bearskin Lake (Minnesota, USA; 48.064°N, 90.403°W) sediment, mixing thoroughly by hand, then placing on a roller mill (2 rpm) under refrigeration for one week prior to use. These concentrations of metal were chosen to exceed the sulfide binding capacity of the sediment, and thus increase bioavailability for accumulation, but not so high as to be toxic to the worms. Worms

were exposed for 21 d in 300 mL beakers (ca. 3 g worms per beaker) to which 100 mL of sediment was added. Beakers were held in a water renewal system (after Zumwalt et al. 1994) at 23 °C and received water equal to three volume additions per day. On the day designated for their use in the fish exposures, beakers were transported to MED where the worms were sieved from the sediments, rinsed, and apportioned to fish rations.

For Experiments 2 and 3, worms were obtained daily from culture and exposed to the desired chemical concentrations in water for 13–15 d in 1 L beakers. Exposure was initiated by adding the following to beakers: 30 mL of a slurry of ground paper towels; 800 mL of the desired exposure solution; ca. 10 g worms; and 4 mL of 25 g·L^{-1} Zeigler® Aquatox (Aquatic Eco-Systems Inc., Apopka, Florida, USA) food slurry. Nominal concentrations were 100 µg Cd·L^{-1} and 150 µg Cd·L^{-1}, 100 µg Cu·L^{-1} and 150 µg Cu·L^{-1}, and 600 µg Pb·L^{-1}. The exposure solution was decanted off daily, and new solution and food slurry were added. The above mixtures were lightly aerated in a 23 °C incubator with a 16 (light):8 (dark) photoperiod. On the day designated for their use, beakers were withdrawn from the incubator and the worms needed for fish rations were placed in vials for transport to MED.

For Experiments 4 to 6, *L. variegatus* were exposed in water for 7 d using a proportional flow diluter (Benoit et al. 1982) configured to deliver uncontaminated water (23 °C) and two concentrations of the test chemical (nominal concentrations 67 µg Cu·L^{-1} and 100 µg Cu·L^{-1} for Experiment 4; 4.0 µg As·L^{-1} and 8.0 µg As·L^{-1} for Experiment 5; and 1.5 µg As·L^{-1} and 3.0 µg As·L^{-1} for Experiment 6). Each day, approximately 2 g of cleaned worms were placed into 300 mL beakers to prepare the diet for use 7 d later, the number of beakers being sufficient to provide the total mass needed for fish rations and analytical samples. The beakers were placed in aquaria receiving the diluter flows; water exchange into and out of the beakers was provided for by screened openings in the sides of the beakers providing for water exchange and a siphon system for the aquaria effluent that fluctuated the water level. On the day designated for their use, beakers were withdrawn from the aquaria, and worms from beakers receiving the same concentration were pooled into a single mass. Water adhering to the worms was removed by placing them on filter paper in a Buchner funnel and applying a gentle vacuum, and desired rations were then apportioned by weight (mass).

Fish dietary exposures

At the start of each test, eight fish were placed in each experimental chamber (rectangular glass tank holding 4.6 L water) receiving 1.6 L·h^{-1} of uncontaminated water, at 25 °C for Experiments 2 and 3 and at 11 °C for other experiments. A separate set of 20–30 fish was individually weighed to obtain a mean and standard error for initial wet mass (Table 1). Mortality was monitored daily. Each chamber received a once-daily ration of worms (by count for Experiments 1–3 and by mass for Experiments 4–6). Depending on initial fish size, the initial ration per fish ranged across experiments from 15%–34% (wet mass food per wet mass fish), and rations were periodically increased to maintain similar percentages based on expected control fish growth, in some cases being slightly reduced due to diet availability. Any uneaten worms from the previous day were removed and weighed prior to introducing the current day's ration. At termination, fish were euthanized, the standard length (nearest millimetre) and wet mass (nearest 0.1 mg) of each fish were measured, and the percent dry mass was determined for a pooled sample of 3–5 fish from each replicate.

For Experiment 6, eight fish from each treatment were submitted to Experimental Pathology Laboratories (EPL), Herndon, Virginia for histological examination. Fish were fixed with Bouin's solution at MED and embedded in paraffin by EPL. Four sagittal sections were prepared from each fish, stained with hematoxylin and eosin, and examined for effects on liver, kidney, gallbladder, stomach, pyloric ceca, and intestine.

Characterization of exposure conditions

The primary exposure variable of interest — the metal/metalloid contamination in the diet — was measured by nitric acid digestion followed by atomic absorption spectrophotometry. Diet samples were collected 2–3 times per week for each treatment, with at least 25% replication. For Experiment 5, the loss of arsenic from the diet was also assessed, and indicated no significant loss would occur before consumption (for metals, loss was not of concern based on separate work by Dawson et al. 2003).

Dietary quality was documented in these experiments based on measurements of percent dry mass, total lipids, and protein. Percent dry mass was based on drying diet samples for 24 h at 90 °C. Protein was analyzed colorimetrically according to Bradford (1976) using the Bio-Rad Laboratories (Hercules, California, USA) assay kit. Lipid was determined by extraction with an acetonitrile–dichloromethane–methanol or hexane–isopropanol mixture and weighing the residue after drying.

Toxicant concentrations used to expose *L. variegatus* were measured by atomic absorption spectrophotometry (after nitric acid digestion for sediment exposures; summarized in Table 1). For arsenic exposures, analyses also included chromatographic separation of arsenite and arsenate, which demonstrated that speciation was not altered in the exposure chambers (data not shown). To determine whether incidental contamination of fish exposure water by leaching from diets or fish excretion was significant, total toxicant concentrations in fish exposure water were measured weekly by atomic absorption spectrophotometry, demonstrating contamination of <1 µg·L^{-1} (data not shown).

In the exposure water for both *L. variegatus* and fish, periodic measurements were made of temperature by portable thermistor meter, dissolved oxygen concentration by membrane oxygen electrode, pH by combination glass electrode, hardness by EDTA titration, alkalinity by potentiometric acid titration, conductivity by coulometry, and total ammonia by ammonium ion specific electrode.

Further sampling and methodological details, quality assurance results, and summaries of test conditions are provided online in Supplemental Appendix S1[3].

[3] Supplemental materials available online from cjfas.nrc.ca.

Data analysis

Individual fish could not be treated as experimental units because they were fed collectively and not ad libitum, so that fish within an experimental chamber were not statistically independent; rather, assessments of effects were based on average values for each experimental chamber. Therefore, the final average wet mass per fish was calculated for each chamber. These were converted to final average dry masses per fish using the average percent dry mass for the treatment.

Final dry masses per fish were converted to food conversion efficiencies by subtracting initial dry mass and dividing by the total dry mass diet consumed per fish. This total dry mass diet per fish was calculated for each chamber by summing the wet masses of diet consumed per fish for each day (= ration size minus unconsumed worm mass collected the next day divided by the number of fish) and multiplying by the average percent diet dry mass for the treatment. Because the ration size per chamber was adjusted for any mortalities to provide a constant ration per fish, this calculation excludes diet provided to the fish that die, to the degree they consumed their share prior to death. Because only wet masses were measured on the samples of the fish collected at the start of each experiment, the percent dry mass for the initial fish needed to be estimated. For rainbow trout, this was based on the relationship of percent dry mass to wet mass for control fish at 15 d and 30 d in Experiments 1, 4, 5, and 6, which ranged from 0.3 g to 1.4 g wet mass (wm). Linear regression analysis was conducted on these data (% dm = 14.7 + 0.041·mg wm, R^2 = 94%) and this relationship was used to estimate the percent dry mass for rainbow trout at the start of each experiment. For channel catfish, the ratio of percent dry mass to wet mass at the end of Experiment 2 agreed closely with the trout relationship, so this relationship was also used to estimate the initial channel catfish percent dry mass. For fathead minnow, the ratio of percent dry mass to wet mass at the end of Experiment 3 deviated substantially from the trout relationship, so new samples of 30, 60, and 90-day-old fish were obtained from MED culture to determine a separate relationship for this species. Although estimating the initial percent dry mass in this manner introduces some uncertainty, the impact on estimated dry mass increases was less than 5% compared with simply using the final percent dry mass, and had no effect at all on the statistical evaluation of treatment effects within experiments because any error in initial dry masses is constant for all treatments.

Within each experiment, effects of treatments on average final wet and dry masses per fish and on conversion efficiencies were analyzed by one-way analysis of variance, using Dunnett's test to test significance of chemical effects versus controls. For conversion efficiencies, this analysis was conducted only for Experiments 4–6, because for Experiments 1–3 conversion efficiencies could only be computed as treatment averages (because ration masses were only available as treatment averages). The effects of treatments on diet composition (percent dry mass, lipid, protein) were also tested by one-way analysis of variance (ANOVA) for each experiment, but to more thoroughly assess such effects, for each chemical, the combined data from all experiments were analyzed with a two-way, main-effects ANOVA, one factor being the experiment and the other being control versus chemical (regardless of concentration) within the experiment. Differences in dietary composition among experiments were also evaluated based on a one-way ANOVA for control data only. All statistical analyses were conducted using the general linear model procedure of Statistica 8.0 (StatSoft Inc., Tulsa, Oklahoma, USA) and statistical significance was based on the probability, p, of a Type I error relative to a null hypothesis of equal means.

Results and discussion

Diet characteristics

No overt effects on *L. variegatus* mortality, appearance, and behavior owing to chemical exposure were observed. However, analyses of *L. variegatus* for percent dry mass, lipid, and protein did show some differences among experiments and treatments (Table 2), warranting consideration of whether such differences might appreciably confound interpretation of the effects of dietary contamination on fish growth.

For *L. variegatus* percent dry mass, treatment means varied from 15.2% to 17.4% (Table 2) with an overall mean of 16.4%. Although the extremes deviate little from the overall mean, there are some apparent trends in the data. Compared with their respective controls, the percent dry masses were always greater for copper-treated worms (by an average of 0.9%) and were always lower for lead-treated worms (by an average of 0.6%), whereas for cadmium- and arsenic-treated worms the average deviation was less than 0.2%. The Dunnett's tests for the one-way ANOVAs of the individual experiments showed $p > 0.10$ for all comparisons of treatments with controls except for two copper treatments (Experiment 4, $p < 0.01$) and one lead treatment (Experiment 1, $p = 0.054$), but by collectively examining data for all experiments, the two-way ANOVAs for each chemical indicated the effects to be significant for copper ($p < 0.01$) and lead ($p = 0.011$). For the higher copper treatment in Experiments 2 and 3, the higher percent dry masses were associated with 10% lower absolute dry masses per worm than the controls, perhaps indicative of poorer worm condition, but dry masses per worm were not significantly affected for any other treatment.

Because such chemical effects on percent dry mass would be expected to affect lipid and protein expressed on a wet mass basis, measurements for these constituents are reported on a dry mass basis, based on the average percent dry mass for the treatment (Table 2). For percent lipid, treatment means varied from 9.8% to 11.8% for Experiments 1–3 and from 17.0% to 18.6% for Experiments 4–6 (Table 1). The difference between these groups of experiments coincides with a change in the source for the worms and in the nature of the worm exposures. A one-way ANOVA across experiments of the control worms showed a significant difference ($p < 0.01$) between these groups of experiments. Although converting to a dry mass basis would correct for the effects of copper on percent dry mass, the percent lipid of copper-treated worms still averaged higher (by 0.8%) than their respective controls, with five of the six treatments being at least 0.4% higher, while no consistent deviations were observed for other chemicals. The Dunnett's tests for the one-

Table 2. Diet composition.

Treatment	Toxicant content[a]	Percent dry mass	Percent lipid (dm basis)	Percent protein (dm basis)
Experiment 1				
Control		16.2	9.8	48.3
Cd	91 μg Cd·(g dm)$^{-1}$ (34%)	15.9	10.6	51.2
Pb	956 μg Pb·(g dm)$^{-1}$ (16%)	15.3*	11.5	45.1
Experiment 2				
Control		15.9	11.4	37.3
Low Cd	369 μg Cd·(g dm)$^{-1}$ (17%)	15.7	10.6	35.4
High Cd	538 μg Cd·(g dm)$^{-1}$ (17%)	15.5	10.4	36.2
Low Cu	157 μg Cu·(g dm)$^{-1}$ (24%)	16.2	11.8	34.4
High Cu	246 μg Cu·(g dm)$^{-1}$ (17%)	16.5	11.3	39.9
Pb	1000 μg Pb·(g dm)$^{-1}$ (13%)	15.2	11.3	36.7
Experiment 3				
Control		16.2	10.4	39.7
Low Cd	356 μg Cd·(g dm)$^{-1}$ (12%)	16.7	10.3	39.9
High Cd	518 μg Cd·(g dm)$^{-1}$ (8%)	16.2	10.8	37.6
Low Cu	131 μg Cu·(g dm)$^{-1}$ (10%)	16.8	11.0	40.8
High Cu	216 μg Cu·(g dm)$^{-1}$ (10%)	17.2	11.8	42.3
Pb	846 μg Pb·(g dm)$^{-1}$ (13%)	16.1	10.4	39.1
Experiment 4				
Control		16.2	17.0	40.4
Low Cu	180 μg Cu·(g dm)$^{-1}$ (18%)	17.4***	17.6	39.9
High Cu	308 μg Cu·(g dm)$^{-1}$ (15%)	17.2***	18.6**	42.1
Experiment 5				
Control		16.4	17.9	36.6
Low arsenate	40 μg As·(g dm)$^{-1}$ (27%)	16.6	17.5	36.9
High arsenate	76 μg As·(g dm)$^{-1}$ (31%)	16.5	17.9	35.7
Experiment 6				
Control		16.8	18.4	36.4
Low arsenite	28 μg As·(g dm)$^{-1}$ (35%)	16.9	17.7	36.8
High arsenite	61 μg As·(g dm)$^{-1}$ (28%)	16.8	17.8	35.5

Note: dm, dry mass. For control treatments, *L. variegatus* toxicant content averaged 12 μg Cu·(g dm)$^{-1}$, 2 μg Cd·(g dm)$^{-1}$, 5 μg Pb·(g dm)$^{-1}$, and 5 μg As·(g dm)$^{-1}$. Asterisks denote Type I (two-tailed) error probability (p) for difference from control; ***, $p < 0.01$; **, $0.01 < p < 0.05$; *, $0.05 < p < 0.10$; no asterisks, $p > 0.10$.

[a]Mean toxicant concentration in diet for $n = 10$ to 30 measurements; values in parentheses are the coefficients of variation expressed as a percentage of the mean.

way ANOVAs of the individual experiments only showed a significant difference for one of the six copper treatments ($p = 0.015$; all other effects $p > 0.10$), but the two-way ANOVA of chemical versus experiment showed the collective copper effect to be significant at the $p = 0.058$ level.

For percent protein, treatment means varied from 45% to 51% for Experiment 1 and from 34% to 42% for Experiments 2–6 (Table 1). This difference between these groups of experiments corresponds with sediment versus water exposure and a one-way ANOVA across experiments of the control worms showed a significant difference ($p < 0.01$) between these groups, possibly indicating better nutrition status from the sediment exposure. Although copper-treated worms again had slightly elevated average protein values relative to the controls, and lead had slightly lower values (Table 1), no chemical effects were significant ($p > 0.10$) by either the one-way ANOVAs of the individual experiments or the two-way ANOVAs for each chemical.

Although the magnitudes of the chemical effects on percent dry mass were not great, they should be taken into account in analyzing effects on growth. For example, when the offered ration is constant on a wet mass basis, the difference for the copper treatments represents about 5% more dry mass of food than the controls. This was the reason for addressing food conversion efficiencies on a dry mass basis in the growth analysis. Even for conversion efficiencies based on dry mass, the higher lipid + protein content for the copper-treated fish also bears some consideration when interpreting fish growth, despite the small magnitude and weak statistical significance.

Fish survival

Fish survival was good in all treatments and showed no relationship to dietary exposure to any of the chemicals. Of the 960 experimental fish, there were only 16 mortalities (1.7%), of which 5 were in controls and 11 were in various

Table 3. Fish growth in relationship to dietary metal/metalloid exposure.

Treatment	Duration(d)	Final wet mass[a] (g·fish^{-1})	Percent dry mass	Final dry mass (g·fish^{-1})	Total ration [(g dm)·fish^{-1}]	Percent food conversion (dm basis)
Experiment 1						
Control	30	0.666 (4.5%)	17.4	0.116	0.296	33
Cd	30	0.596 (13%)	17.2	0.103	0.289	29
Pb	30	0.709 (1.7%)	17.8	0.126	0.272	40
Experiment 2						
Control	30	0.807 (3.7%)	17.9	0.144	0.383	30
Low Cd	30	0.825 (3.6)	17.6	0.145	0.388	29
High Cd	30	0.818 (5.0%)	17.8	0.146	0.376	30
Low Cu	30	0.845 (6.4%)	18.3	0.154	0.348	34
High Cu	30	0.802 (3.1%)	17.5	0.140	0.327	34
Pb	30	0.845 (4.4%)	18.0	0.152	0.410	29
Experiment 3						
Control	30	0.618 (3.6%)	21.1	0.131	0.321	34
Low Cd	30	0.625 (2.7%)	20.9	0.131	0.343	31
High Cd	30	0.601 (3.0%)	20.8	0.125	0.336	30
Low Cu	30	0.666 (2.7%)**	21.2	0.141**	0.336	36
High Cu	30	0.688 (5.1%)***	22.3	0.153***	0.301	43
Pb	30	0.642 (4.8%)	21.6	0.139	0.367	32
Experiment 4						
Control	30	1.215 (1.6%)	19.4	0.235	0.483	35
Low Cu	30	1.378 (5.7%)***	20.2***	0.279***	0.520	41**
High Cu	30	1.302 (1.8%)*	20.5***	0.267***	0.513	39**
Experiment 5						
Control	15	0.350 (2.0%)	16.8	0.059	0.086	42
Low arsenate	15	0.319 (1.0%)***	15.7***	0.050***	0.086	32***
High arsenate	15	0.296 (5.7%)***	15.2***	0.045***	0.086	27***
Control	30	0.728 (3.8%)	18.1	0.131	0.269	41
Low arsenate	30	0.563 (11%)***	16.7***	0.094***	0.268	27***
High arsenate	30	0.516 (4.3%)***	16.2***	0.084***	0.268	24***
Experiment 6						
Control	15	0.477 (9.2%)	16.6	0.079	0.125	34
Low arsenite	15	0.466 (3.9%)	16.3	0.076	0.125	31
High arsenite	15	0.437 (5.3%)*	16.1*	0.070*	0.125	27*
Control	30	0.956 (3.8%)	18.7	0.179	0.360	39
Low arsenite	30	0.885 (7.9%)*	17.7***	0.156*	0.360	33**
High arsenite	30	0.779 (13%)**	17.1***	0.133***	0.360	28***

Note: dm, dry mass. Asterisks denote Type I (two-tailed) error probability (p) for difference from control; ***, $p < 0.01$; **, $0.01 < p < 0.05$; *, $0.05 < p < 0.10$; no asterisks, $p > 0.10$.

[a]Mean fish mass over all four replicate tanks; parentheses denote coefficient of variation among means for individual tanks as a percentage of overall mean.

chemical treatments. Of the 120 experimental chambers, 105 had 8 survivors (100% survival), 14 had 7 survivors, and 1 had 6 survivors.

Effects of metals on fish food consumption and growth

For dietary metal exposures (Experiments 1–4), growth on either a wet- and dry-mass basis was not adversely affected by any treatment (Table 3) and the fish consumed >99% of the offered rations. For rainbow trout and fathead minnow, growth was actually significantly better for copper-contaminated diets, and conversion efficiencies were also the highest for the copper treatments (Table 3). For channel catfish, final masses averaged only slightly higher in the copper treatments, but this was despite the dry mass of diet being less than the controls, so that copper again was associated with the highest conversion efficiencies. Over all three fish species, conversion efficiencies for copper treatments averaged 38% versus 33% for the controls, which was significantly higher ($p < 0.01$) based on a paired t test. Because these efficiencies are on a dry mass basis and because the increase is too large to be reasonably attributed to diet quality or quantity (Tables 2 and 3), other positive effects of copper on fish growth (e.g., its role as an essential micronutrient) must be considered as possible explanations.

For rainbow trout exposed to copper in their diet, the absence of adverse effects in the current study, for dietary levels up to 308 μg Cu·(g dm)$^{-1}$ (Tables 2 and 3) is consistent with several studies with metal-salt contaminated diets (see Clearwater et al. 2002; Borgmann et al. 2005) and copper-contaminated brine shrimp (Mount et al. 1994), which col-

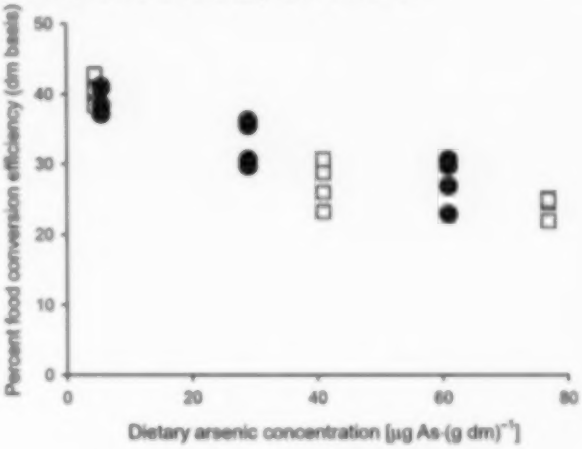
lectively suggest that adverse effects on growth first appear at 500–700 μg Cu·(g dm diet)$^{-1}$. The results of the current study therefore do not support concerns that metal-salt contaminated diets grossly underestimate risk. Clearwater et al. (2002) noted that dietary exposures might be best compared on a dose basis that accounts for the feeding rate, and provided a meta-analysis of past studies that indicated significant growth effects required 35–45 μg Cu·(g wm fish)$^{-1}$·d^{-1}. This is also above the dose in the current study, which was in the range of 6 μg Cu·(g wm fish)$^{-1}$·d^{-1}.

For copper exposures to channel catfish, the results in the current study indicated no growth effects up to 246 μg Cu·(g dm)$^{-1}$ in the diet, in contrast to the 16 μg Cu·(g dm)$^{-1}$ dietary concentration reported to cause growth effects by Murai et al. (1981). On a dose basis, the current study showed no effects up to 5 μg Cu·(g wm fish)$^{-1}$·d^{-1}, again in contrast to 0.4 μg Cu·(g wm fish)$^{-1}$·d^{-1} calculated by Clearwater et al. (2002) from the Murai et al. (1981) data. It should be noted that the results from Murai et al. (1981) are also at odds with those of Gatlin and Wilson (1986), who reported no adverse effects on channel catfish growth at dietary concentrations up to the maximum they tested [40 μg Cu·(g dm)$^{-1}$]. An explanation for these large discrepancies is not obvious and warrants further investigation, with one factor to consider being that Murai et al. (1981) did not document actual consumption, and different diet preparations might differ in palatability.

For lead, the current study showed no effects at 856–1000 μg Pb·(g dm diet)$^{-1}$ for all three fish species (Tables 2 and 3). Previous studies with dietary lead exposure in rainbow trout have shown no effects on growth up to maximum tested concentrations of 100 μg Pb·(g dm)$^{-1}$ (Hodson et al. 1978), 210 μg Pb·(g dm)$^{-1}$ (Mount et al. 1994), and 495 μg Pb·(g dm)$^{-1}$ (Alves and Wood 2006; Alves et al. 2006). Limited, transient effects on certain physiological parameters (hematocrit, blood ions) were observed at concentrations at-and-below these maxima (Hodson et al. 1978; Alves and Wood 2006; Alves et al. 2006), but are thus apparently not closely correlated with, or suitable indicators of, effects on growth.

For cadmium, the current study showed no effects on growth of channel catfish and fathead minnow at dietary levels of ca. 500 μg Cd·(g dm)$^{-1}$, or rainbow trout at 91 μg Cd·(g dm)$^{-1}$ (Tables 2 and 3). Previous reports have shown that dietary cadmium had no effect on the growth of rainbow trout, up to maximum tested concentrations in the diet of 70 μg Cd·(g dm)$^{-1}$ (60 d, Mount et al. 1994) and 300 μg Cd·(g dm)$^{-1}$ (30 d, Baldisserotto et al. 2005). For another salmonid, Atlantic salmon (*Salmo salar*), Berntssen and Lundebye (2001) reported no effect on growth over 16 weeks from exposure to 250 μg Cd·(g dm)$^{-1}$ in the diet, although body compositional changes were noted at 125 μg Cd·(g dm)$^{-1}$ and above, which, again, are apparently not close correlates of growth effects. Using a live diet of oligochaetes, similar to the current study, Ng and Wood (2008) reported reduced rainbow trout growth rates at dietary levels of cadmium of 30 μg Cd·(g wm diet)$^{-1}$ (about 180 μg Cd·(g dm diet)$^{-1}$) during the last 2 weeks of a 4-week exposure, but not at 2.2 μg Cd·(g wm diet)$^{-1}$ (about 13 μg Cd·(g dm diet)$^{-1}$). This is not inconsistent with the absence of effects on rainbow trout observed in the current study over the entire 30 d dietary exposure at 91 μg Cd·(g dm)$^{-1}$. However, Ng et al. (2009) reported growth reductions in rainbow trout at much lower exposures — 12 μg Cd·(g dm diet)$^{-1}$ — in the last week of a 4 week exposure, for a pelletized diet of ground oligochactes amended with cadmium nitrate. This inconsistency between Ng et al. (2009) and both Ng and Wood (2008) and the current study warrants further investigation, especially regarding possible effects of the method for preparing the diet and the timeframe for monitoring growth.

Fig. 1. Percent food conversion efficiency of juvenile rainbow trout versus dietary arsenic concentration. Filled circles denote tanks in which fish were fed *Lumbriculus variegatus* that had been exposed to arsenite; open squares denote tanks in which fish were fed *L. variegatus* that had been exposed to arsenate.

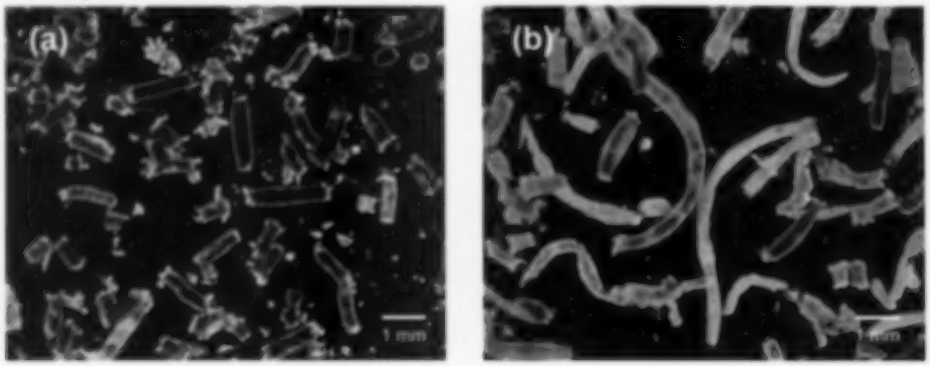


These results of the current and past studies suggest that toxicity due to copper, cadmium, or lead is not a likely cause for the growth effects reported by Woodward et al. (1994, 1995). CFR-derived invertebrate diets which caused reductions in growth contained 0.3–3.2 μg Cd·(g dm)$^{-1}$, 15–33 μg Pb·(g dm)$^{-1}$, and 100–415 μg Cu·(g dm)$^{-1}$. These cadmium concentrations are much less even than the exposures of Ng and Wood (2008) and Ng et al. (2009). Similarly, these lead concentrations are many times less than no-observable effects concentrations (NOECs) observed in the current and past laboratory studies. The copper concentrations in the diets of Woodward et al. (1994, 1995) also would be expected to be NOECs, but the highest copper concentration in these diets was within a factor of two of expected effects concentrations, so copper might be a contributor to toxicity, provided there are other toxicants at or near their effect concentration range which would have joint effects with copper.

Effects of arsenic on fish food consumption, growth, and histology

In contrast to the tested metals, dietary arsenic exposures (Experiments 5–6) caused reductions in rainbow trout final masses for all treatments (Table 3), with reductions being significant, at least at the $p = 0.10$ level, except for the 15 d exposure at the lowest diet concentration (the lower arsenite exposure). A 30 d NOEC cannot be assigned based on this data, other than to say that it is likely less than 29 μg As·(g dm diet)$^{-1}$ (0.35 μg As·(g wm fish)$^{-1}$·d^{-1}). One notable as-

Fig. 2. Fecal pellets for (*a*) fish fed *Lumbriculus variegatus* that had been exposed to control water, and (*b*) for fish fed *L. variegatus* that had been exposed to 3 mg·L^{-1} arsenite.



pect of the reduced growth is that whether arsenate or arsenite was used to expose the oligochaete worms did not make an appreciable difference to the relationship of conversion efficiencies to dietary arsenic concentration (Fig. 1). This might reflect speciation changes of arsenic incorporated into the oligochaete diet, which is the subject of ongoing investigation.

These exposures also resulted in reduced consumption of the provided rations. For the 30 d exposures, the control fish consumed >99% of the provided rations, but the fraction uneaten averaged (among replicate tanks) 3.8% in the lower arsenate treatment, 7.1% in the higher arsenate treatment, 1.3% in the lower arsenite treatment, and 4.9% in the higher arsenite treatment. This reduced food consumption was also accompanied by much slower feeding. Whereas control fish would typically consume the daily ration in less than 0.5 h, consumption of the arsenic-treated worms would often extend much longer. The uneaten diet contributed only partly to the reduced growth, because the reduction in consumption was much less than the reduction in fish growth (Table 3), although at higher feeding rates, the slower consumption would presumably assume greater importance. That growth effects were not just a matter of reduced consumption is also evidenced in food conversion efficiencies being significantly reduced (Table 3; Fig. 1).

Associated with the reduced and slower consumption of arsenic-contaminated worms compared with control worms was a difference in the appearance of the fish fecal matter. For control diets, fecal pellets were predominantly dark and compact (Fig. 2*a*). In contrast, fecal matter for arsenic treatments was typically more lightly colored and less well formed (Fig. 2*b*), indicative of some disruption of digestion.

These arsenic effects on growth and food consumption are generally consistent with past studies using arsenate-salt amended diets. For one experiment, Cockell et al. (1991) reported no effects on growth and food consumption of juvenile rainbow trout (8 g initial mass) at 8 μg As·(g dm diet)$^{-1}$, a 45% reduction in food consumption and 55% reduction in growth at 44 μg As·(g dm diet)$^{-1}$, and >85% reduction in food consumption and cessation of growth at 104 μg As·(g dm diet)$^{-1}$ and above. In contrast to the current study, these arsenic effects on growth were largely attributable to reduced food consumption. This difference between the studies might be attributable to the fact that Cockell et al. (1991) fed their fish ad libitum for a fixed period, whereas the current study used a fixed ration which, as noted above, was consumed over a longer time for the arsenic treatments than the controls. In a second experiment, Cockell et al. (1991) limited consumption rates in additional control treatments to that in the arsenic treatments, and thereby also documented arsenic-induced reductions in food conversions in addition to the reduced consumption. At 49 μg As·(g dm)$^{-1}$ in diet, the food conversion (fish wet mass gain per dry mass diet) was 0.98 versus 1.09 in the consumption-matched control (10% reduction) and at 182 μg As·(g dm diet)$^{-1}$ the difference was 0.42 versus 0.71 (40% reduction). For a 64 d exposure to a different salmonid, lake whitefish, Pedlar et al. (2002) reported food refusal (partial starting at day 45 and complete at day 55) and reduced (67%) growth rate at 100 μg As·(g dm diet)$^{-1}$, but not at 10 μg As·(g dm diet)$^{-1}$ and below. Although the concordance of effect concentrations between these past studies and the current study suggests that biological incorporation of arsenic had little effect on toxic potency, this applies only to aqueous exposure of *L. variegatus* to inorganic arsenic.

Histological examination of fish from Experiment 6 revealed dose-related effects in the liver. Anisokaryosis (differences in sizes of cell nuclei) of hepatocytes was not observed in control fish, but was found in 2 of 8 fish from the low arsenite treatment, and in all 8 fish in the high arsenite treatment. Microvesicular vacuolation, indicative of poorer nutritional status, was absent in control fish, but present in 1 of 8 fish from the low arsenite treatment, and 5 of 8 fish in the high arsenite treatment (liver photomicrographs for a control and treated fish illustrating these effects are included online in Supplemental Figure S1[3]). Cockell et al. (1991) reported no histological effects in the livers of rainbow trout for treatments up to 182 μg As·(g dm diet)$^{-1}$, but this was for macroscopic examination only. For lake whitefish, Pedlar et al. (2002) observed even more severe liver histopathology, including nuclear and structural alterations, inflammation, and necrotic lesions, at exposures as low as 10 μg As·(g dm diet)$^{-1}$.

No dose-related effects were observed in the current study for the stomach, pyloric caecae, stomach, intestine, gall bladder, and kidney. The absence of effects in the gall bladder is in contrast to earlier studies. Cockell et al. (1991) consistently observed lesions in the gall bladder of rainbow trout at exposures of 33 μg As·(g dm diet)$^{-1}$ and above;

however, these effects were observed in exposures of 12–24 weeks, and might not have had time to develop in the 30 d exposures in the current study. For lake whitefish, Pedlar et al. (2002) found similar effects at exposures as low as 10 μg As·(g dm diet)$^{-1}$ and as short as 10 d, noting that this species appeared to be more sensitive than rainbow trout regarding these effects.

These results of the current and past studies suggest that arsenic is more likely a cause of the growth effects reported by Woodward et al. (1994, 1995) and Hansen et al. (2004) than are metals. The CFR-derived invertebrate diets of Woodward et al. (1994, 1995) showed reduced growth from diets containing 19–43 μg As·(g dm)$^{-1}$, which is in the range found to cause growth effects in the current study and past studies with arsenic salts, although the uncertain influence of nutritional differences in the Woodward et al. (1994, 1995) studies on their observed growth effects should also be considered. Hansen et al. (2004), who used sediments from the CFR and other sites to contaminate oligochaetes, reported growth was significantly reduced in rainbow trout fed oligochaetes containing an average 79–129 μg As·(g dm)$^{-1}$. Hansen et al. (2004) did note a correlation of effects to arsenic levels, but by itself this correlation was not meaningful because similar correlations would have existed for other contaminants.

Next steps

This study confirmed that arsenic is a contaminant of concern for fish fed live diets at environmentally relevant concentrations, and therefore is a subject of continuing investigation by the authors. Because arsenic can exist in a variety of chemical forms (different oxidation states, various organoarsenicals) and because these forms have different toxicities (e.g., Cockell et al. 1991), there is a need to document arsenic speciation in fish prey items in natural systems and to conduct laboratory toxicity tests relevant to this speciation. There is also a need to extend the understanding of dietary arsenic toxicity to other fish species, dietary matrices, and endpoints, especially reproduction and growth under longer exposures. Additionally, the relative importance of water and dietary exposure needs to be determined, especially for situations in which dietary contamination is driven by arsenic contamination in the water column, rather than sediments. In such a case, will waterborne exposure produce substantial toxicity at concentrations lower than that needed to contaminate diets sufficiently to be toxic, making dietary exposure of little importance?

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Genetic homogeneity of weathervane scallops (*Patinopecten caurinus*) in the northeastern Pacific

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Abstract: We assessed genetic differentiation among populations of weathervane scallop (*Patinopecten caurinus*) in the northeastern Pacific, extending over 2500 km in the Gulf of Alaska and southeastern Bering Sea. Variability was surveyed at nuclear loci with allozyme, microsatellite, and single nucleotide polymorphism (SNP) methods, and at mitochondrial (mt)DNA loci with SNPs and nucleotide sequencing. High levels of gene diversity were detected for allozymes (H = 0.080), microsatellites (H = 0.734), and mtDNA (h = 0.781). Genotypes at nuclear loci generally fit Hardy–Weinberg proportions, except for some microsatellite loci, for which null-allele frequencies of 0.02 to 0.34 were estimated. No allele-frequency differences were detected among samples, except for the allozyme loci *Gpi* and *Pep-4*. Overall levels of differentiation ranged from F_{ST} = 0.0004 for allozymes, F_{ST} = 0.0008 for mtDNA to F_{ST} = 0.0004 for microsatellites. No isolation by distance was found for any of the markers. A unimodal mtDNA mismatch distribution and significant excesses of low-frequency variants for allozymes, microsatellites, and mtDNA may reflect a post-glacial population expansion. The lack of genetic differentiation measured by neutral markers does not preclude the existence of locally adapted, self-sustaining populations that are important in the harvest management of this species.

Résumé : Nous avons évalué la différenciation génétique dans des populations du pétoncle géant du Pacifique, *Patinopecten caurinus*, dans le nord-est du Pacifique sur plus de 2500 km dans le golfe de l'Alaska et le sud-est de la mer de Béring. La variabilité des locus nucléaires a été déterminée par les méthodes des allozymes, des microsatellites et du polymorphisme mononucléotidique (SNP) et celle des locus d'ADNmt mitochondrial par les méthodes de SNP et de séquençage des nucléotides. De fortes valeurs de diversité génique sont décelables chez les allozymes (H = 0,080), les microsatellites (H = 0,734) et l'ADNmt (h = 0,781). Les génotypes aux locus nucléaires suivent généralement les proportions de Hardy–Weinberg, à l'exception de certains locus microsatellites chez lesquels des fréquences d'allèles nuls de 0,02 à 0,34 ont été estimées. Il n'y a pas de différences de fréquences d'allèles entre les échantillons, excepté pour les locus d'allozymes *Gpi* et *Pep-4*. Les valeurs globales de différenciation vont de F_{ST} = 0,0004 pour les allozymes et F_{ST} = 0,0008 pour l'ADNmt à F_{ST} = 0,0004 pour les microsatellites. Aucun isolement par distance ne se retrouve chez les marqueurs. Une distribution des différences (mismatch distribution) unimodale de l'ADNmt et des surplus significatifs des variantes de faible fréquence des allozymes, des microsatellites et de l'ADNmt peuvent être le reflet d'une expansion postglaciaire des populations. L'absence de différenciation génétique mesurée à l'aide des marqueurs neutres n'exclut pas l'existence de populations adaptées localement et autosuffisantes qui sont importantes dans la gestion de la récolte chez cette espèce.

[Traduit par la Rédaction]

Introduction

The identification of biologically meaningful population boundaries for stock assessment and harvest management is an enduring challenge to fishery managers. Marine invertebrates generally have large population sizes, high fecundities, and planktonic larvae that encounter few barriers to dispersal in the open ocean. Hence, high levels of gene flow are expected to produce genetic homogeneity among populations (Waples 1998). On the other hand, larval retention by ocean gyres and selection may tend to isolate populations and produce genetic differences between groups. On

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decadal time scales, climate change also influences population structure by forcing shifts in the distributions of populations into optimal habitats (MacCall 1990). Additionally, the effects of ice-age shifts in species' distributions may have influenced large-scale patterns of differentiation among contemporary populations.

This study focuses on weathervane scallops, *Patinopecten caurinus*, which inhabit subpolar to temperate coastal waters extending 5000 km from the Pribilof Islands in the Bering Sea, to Point Sur, California (Jablonski and Valentine 1990; Foster 1991). In Alaska, scallops are harvested by a small commercial fleet (Foster 1991; Kruse et al. 2005). The continuing expansion of Alaska's scallop fishery (Shirley and Kruse 1995) and the species' long lifespan (Ignell and Haynes 2000) expose weathervane scallops to possible overexploitation. Concentrations of scallops occur on firm, sandy and muddy substrates, which are found intermittently in the shallow coastal areas of the Gulf of Alaska and southeastern Bering Sea (Masuda and Stone 2003). This patchy distribution complicates harvest management, because it is unknown whether the availability of suitable patchy habitats, oceanographic features, or life history limits local recruitment and abundance. In the absence of evidence for genetic stock structure, scallop culture and harvest are regulated in two ways. First, the State of Alaska has designated six "larval drift zones" to manage the transport of individuals for culture and release (www.cf.adfg.state.ak.us/geninfo/enhance/mariculture/larval_drift_zones.pdf). Second, the North Pacific Fishery Management Council regulates harvests in nine regulatory areas that do not entirely match the larval drift zones (The Scallop Plan Team 2009).

Several factors may influence population structure in scallops. Individuals inhabit depths from the intertidal zone to about 300 m, but are most abundant at 40–130 m. Adults are dioecious (unlike most other pectinids), and external fertilization is followed by planktonic larval development lasting a few weeks. During this time, coastal and offshore currents can potentially move larvae long distances. After settlement, individuals usually mature in 3–4 years, depending on habitat, and grow to large sizes (20–24 cm) (Kruse et al. 2005). Some life history differences among populations may indicate demographically independent populations. The patchy distribution of suitable habitats may contribute to isolation among populations. Growth rates appear to increase from eastern to western areas in the Gulf of Alaska (Ignell and Haynes 2000; Kruse et al. 2005), but it is not clear whether these differences reflect genetic or environmental differences among populations.

Scallop populations in Alaska may also show residual effects of post-glacial expansions, which may still be apparent after hundreds of generations (Hewitt 2000). These effects may be detected by departures from mutation-drift equilibrium. A population bottleneck during a glacial maximum leads to the loss of low-frequency alleles and to heterozygosity excesses (distinguished from Hardy–Weinberg heterozygote excess) relative to populations in equilibrium (Luikart et al. 1998). As populations rebound, new mutations accumulate and produce excesses of low-frequency alleles (heterozygosity deficiency) for about $0.4N_e$ generations (Maruyama and Fuerst 1984). Large populations experience proportionally greater losses of alleles after a bottleneck (Ryman et al. 1995) and show greater excesses of low-frequency alleles during recovery (Maruyama and Fuerst 1984). The mutation-frequency spectrum asymptotically approaches drift-mutation equilibrium after about $2N_e$ generations, as low-frequency mutations drift to intermediate frequencies. Several statistics can be used to detect these disequilibria (Tajima 1989; Fu 1997; Reich et al. 1999). Ice-age disequilibria must be considered in an assessment of scallop population structure with genetic markers.

If scallop populations are genetically distinct from one another, and by inference largely self-recruiting, emphasis must be placed on monitoring and managing individual populations. However, if populations are genetically homogeneous, the degree of self-recruitment is uncertain; genetic homogeneity may reflect high levels of gene flow mediated by external recruitment (Waples 1998) or may characterize isolated populations derived by post-glacial colonization from a single refuge. In this case, demographic and ecological information may provide more guidance for management than genetic data. The goal of this study was to use allozymes, microsatellites, and single-nucleotide polymorphisms (SNPs) in nuclear and mitochondrial (mt) DNA to examine the genetic population structure of weathervane scallops, with the goal of helping to define management units.

Materials and methods

Samples and molecular markers

Scallops were collected at eight localities in the Bering Sea and Gulf of Alaska (Table 1; Fig. 1). Tissue samples were archived at –80 °C at the Alaska Department of Fish and Game (ADF&G) in Anchorage, Alaska until processed. Standard starch-gel allozyme electrophoresis of aqueous extracts of adductor muscles from two populations (BERS and YAKU) resolved 35 allozyme loci: *Ah*, *Gpi*, *Pep* (five loci), *Pgm* (two loci), *Aat* (two loci), *Ak*, *Ark* (two loci), *βGal*, *Est* (two loci), *Fdgh*, *Gapdh*, *G3pdh*, *Iddh*, *IdhP* (three loci), *Ldh*, *Mdh* (two loci), *MeP* (two loci), *Mpi*, *Odh*, *Pgdh*, *Pnp*, *Sod*, and *Tpi*. Of these, eight polymorphic loci (*Ah*, *Gpi*, *Pep-1*, *Pep-2*, *Pep-3*, *Pep-4*, *Pgm-2*, and *Pgm-3*) were examined in six samples (BERS, SHUM, SHEL, NEKO, KAYA, YAKU).

Fifteen of 16 microsatellite loci (Elfstrom et al. 2005*b*) were examined in eight samples, with mean locus sample sizes ranging from 143 to 183 scallops. This set of markers comprised 5 dinucleotide, 5 trinucleotide, and 5 tetranucleotide loci.

Previously developed SNPs were assayed by high-throughput methods (Elfstrom et al. 2005*a*) and included nine mtDNA polymorphisms and three nuclear SNPs (one in octopine dehydrogenase (ODH, AY704172:189) and two in a calmodulin-like protein (CaM, AY769248: 282 and 386). SNPs in coding regions were synonymous.

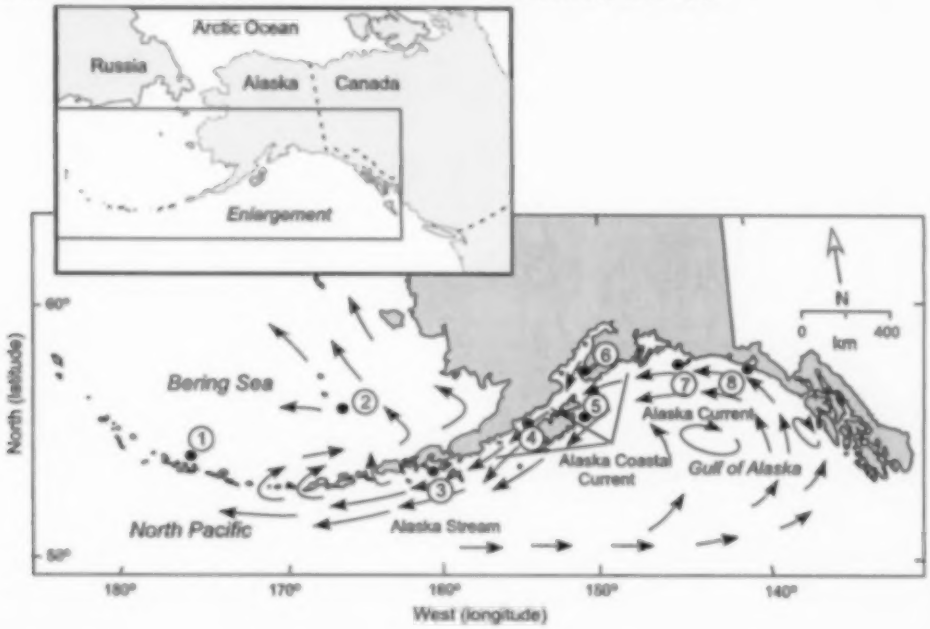
Statistical analyses of nuclear loci

Haplotypes of nuclear amplicons with multiple SNPs were inferred using PHASE 2.1 (Stephens et al. 2001; Stephens and Donnelly 2003). Fit to Hardy–Weinberg expectations (HWE) was tested with Markov chain exact tests (Guo and Thompson 1992), implemented in GENEPOP 4.0 (Rousset 2008). GENEPOP was also used to test for departures from HWE as measured by F_{IS} (Weir and Cockerham 1984) and

Table 1. Dates and locations of weathervane scallop sample collections.

Location	Abbreviation	*N*	Date	N lat., W long.
1. Adak Island	ADAK	300	2002	51°55′, 176°30′
2. Bering Sea	BERS	300	2001	53°50′, 168°00′
3. Shumigan Islands	SHUM	234	2001	55°00′, 159°40′
4. Shelikof Strait	SHEL	300	2001	57°15′, 155°00′
5. NE Kodiak Island	NEKO	300	2001	57°42′, 152°24′
6. Kamishak Bay	KAMI	300	2002	59°35′, 151°45′
7. Kayak Island	KAYA	300	2002	59°55′, 144°32′
8. Yakutat Bay	YAKU	300	2001	59°45′, 139°55′

Fig. 1. Sampling locations for *Patinopecten caurinus* and major currents systems in the Gulf of Alaska and southeastern Bering Sea. Locations: ADAK (1), BERS (2), SHUM (3), SHEL (4), NEKO (5), KAMI (6), KAYA (7), YAKU (8).



to make Monte Carlo R × C tests of allelic-frequency heterogeneity. Tests for correlations between $F_{ST}/(1-F_{ST})$ and geographical distances between samples were made with Mantel's test with 1000 permutations. Geographic distances followed shortest water distances between sample localities. Frequencies of microsatellite null alleles were estimated with ML-NULLFREQ (Kalinowski and Taper 2006). We used the false discovery rate (FDR) procedure of Benjamini and Yekutieli (2001) (B–Y) as presented by Narum (2006) to minimize statistical artifacts in evaluating tables of *P*-values. POWSIM 4.0 (Ryman and Palm 2006) was used to evaluate statistical power for estimating genetic heterogeneity (F_{ST}).

Two procedures were used to test for mutation-drift equilibrium. Allozyme and microsatellite allele-frequency spectra were tested for fit to equilibrium (Ewens 1972; Watterson 1984) with BOTTLENECK 1.2.02 (Piry et al. 1999). Tests were based on large sample sizes (allozymes, $N = 200$; microsatellites, $N = 172–382$) and on 15–21 loci, well above minimal values needed to provide reasonable power to detect departures from evolutionary equilibrium. The infinite alleles model (IAM) was used to examine allozyme data, and the two-phase mutation model (TPM) was used to examine microsatellite with a 90% chance of a stepwise mutation and a 15% variance. Expected (Hardy–Weinberg) heterozygosity ($H_E = 1-\Sigma p^2$, where the p is allele frequency) and heterozygosity expected at mutation-drift equilibrium (H_{EQ}) were computed with coalescent simulations, conditioned on numbers of alleles and sample sizes. At equilibrium, the chance of finding heterozygosity excess or deficiency at a locus is 50%; $H_{EQ}/H_E < 1$ may indicate a recent population bottleneck and a ratio > 1 may indicate a subsequent population expansion. Evolutionary heterozygosity (H_{EQ}) excesses or deficiencies are distinct from HWE heterozygote excesses or deficits. Tests for heterozygosity deficiency (excess of low frequency alleles) are not biased by the presence of null alleles. Significant departures from a ratio of 1 were tested with a one-tailed Wilcox's signed-ranks test. Evolutionary equilibrium for microsatellite loci was also tested with the intralocus variance k and interlocus g tests of Reich et al. (1999) with KGTESTS (Bilgin 2007).

Gene flow between populations was estimated in two

ways. First, an average level of gene flow was estimated with private alleles with GENEPOP. Second, the maximum likelihood coalescent methods in MIGRATE 2.1.2 (Beerli and Felsenstein 2001; Beerli 2004) provided gene flow estimates between each pair of populations. Parameters, $\Theta = 2N_e2\mu$ (for mtDNA $\Theta = N_e2\mu$) and $M = m/\mu$, were estimated with three short chains of 10 000 trees and two long chains of 100 000 trees with a burn-in of 10 000 trees. M measures the relative importance of migration to mutation in introducing new variants into a population. For nuclear loci, $Nm = \Theta M/4$ estimates the number of migrants between populations in one direction.

Statistical analyses of mitochondrial DNA variability

Two sets of mtDNA data were examined. One set consisted of sequences for 25 individuals. A second mtDNA dataset was reconstructed from mtDNA SNP polymorphisms and used to estimate haplotype (h) and nucleotide (Θ_π) diversities, to conduct Fu's F_S (1997) and Tajima's D_T (1989) tests of neutrality, and to calculate haplotype mismatch distributions with ARLEQUIN 3.01 (Excoffier et al. 2005). Genealogical relationships among haplotypes were depicted by a median joining network (Bandelt et al. 1999) with NETWORK 4.5.0.0 (www.fluxus-engineering.com). Weighted (mutation rate and ti/tv ratio) and un-weighted nucleotide sites produced the same shallow network. Variance components of haplotype and haplogroup variability among samples were estimated with the analysis of molecular variance (AMOVA) in ARLEQUIN. Sequence divergences between haplotypes for AMOVA were measured by the number of nucleotide differences between haplotypes. The total variance was partitioned among and within the regional populations with 10 000 bootstrap re-samplings to establish significance. Migration rates between populations were estimated as above with MIGRATE, but with $Nm = \Theta M/2$ for mtDNA.

Test for a post-glacial expansion was made with mtDNA mismatch analysis (Ray et al. 2003). Fit of the mismatch distribution to a unimodal curve, reflecting population expansion or a selective sweep, was tested with Rogers' raggedness coefficient and with a least squares statistic in ARLEQUIN. The statistic τ, a measure of the accumulation of mutations, was used to estimate time since population expansion began (t) with the relationship $t = \tau/2u$, where u is the mutation rate across the entire DNA sequence (Rogers and Harpending 1992). This mutation rate was estimated by $m_t\mu$, where m_t is the number of base pairs in the sequence, and μ is the per nucleotide site mutation rate. Since coding and non-coding sequences were concatenated for this analysis, 2% and 10% sequence divergences were used to bracket possible mutation rates. Female, effective population sizes before and after population expansion were estimated from Θ_0 and Θ_1, with the theoretical relationship $\Theta = N_e2\mu$. Mutation rates were bracketed to account for calibration error.

Results

Allozymes

Six polymorphic loci (*Ah*, *Gpi*, *Pep-1*, *Pep-3*, *Pep-4*, *Pgm-2*) had most-common allele frequencies < 0.95 (Appendix A) and were surveyed in six samples to estimate population parameters. Significant departures from HWE appeared in BERS and SHEL for *Gpi* using the B–Y method to evaluate 36 tests with table-wide $\alpha' = 0.05$. Pooled *Gpi* genotype frequencies showed a highly significant departure from HWE ($P < 0.00005$), but not a significant inbreeding index ($F_{IS} = 0.008$). Pooled *Pgm-2* genotypes exhibited a significant heterozygote deficit ($F_{IS} = 0.039$, $P = 0.0063$, compared to a critical level of 0.0184 for a table-wide $\alpha = 0.05$).

Exact tests detected allele-frequency differences among samples for *Gpi* ($P = 0.0008$) and *Pep-4* ($P = 0.012$). Heterogeneity for *Pep-1* and *Pep-3* was nearly significant ($P = 0.041$ and 0.047, respectively). Fisher's test of combined P-values across the six loci was significant ($P = 0.0001$). The overall level of divergence among samples was low ($F_{ST} = 0.0004$, range –0.0007 to 0.0011). Nevertheless, pairwise comparisons of samples across all loci showed five significant comparisons after the B–Y modification of probabilities. No significant association appeared between pairwise F_{ST} and geographic distance ($P = 0.58$). The private-allele estimate of pairwise migration was $Nm = 24.4$. Coalescence-based estimates of migrants from MIGRATE ranged from 2.5 (YAKU to NEKO) to 8.9 (NEKO to BERS) individuals per generation, but the pattern of donor-recipient relationships did not show a geographical pattern (data not shown).

Variability at 35 loci was surveyed in two samples (BERS, YAKU), separated by about 2000 km of shoreline distance, to test for departures from the Ewens–Watterson allele-frequency distribution. Expected heterozygosity averaged $H_e = 0.080$ in these two samples and was significantly less than the expected equilibrium heterozygosity ($H_{eq} = 0.138$) in both samples (Wilcox rank test of heterozygosity distributions among loci $P < 0.004$) (Table 2).

Microsatellites

All 15 loci were highly polymorphic, with 9–57 alleles per locus (Table 3). The average number of alleles per sample was largest in eastern Gulf of Alaska samples ($A = 18.5$) and smallest in ADAK ($A = 16.9$) (Table 2). Highly significant heterozygote deficits were observed at 8 loci with null-allele frequencies ranging from 0.01 to 0.34 (Table 3). No allele-frequency heterogeneity was detected among samples before or after adjusting allelic frequencies for null alleles. Overall divergence among samples was similar to allozyme divergence, $F_{ST} = 0.004$ (range: –0.0005 to 0.0011), and no isolation by distance was detected ($P = 0.958$). The private-allele estimate of the number of gene flow was $Nm = 33.2$ and was similar to the average number of migrants into a population, as estimated with coalescence. Coalescent estimates of migration ranged from 2.0 (BERS to ADAK) to 3.8 (SHEL to SHUM) individuals per generation. Patterns of gene flow did not show a geographical pattern. POWSIM analyses indicated that the sampling design was capable of detecting significant population heterogeneity as low as $F_{ST} = 0.0003$ with at least 92% power.

Two tests provided evidence of mutation-drift disequilibrium. H_E ranged from 0.725 to 0.740, but did not show a geographical trend. In each sample, H_{EQ} was significantly greater than H_E, consistent with an historical population expansion (Table 2).

The intralocus k test was highly significant for all samples, with negative k values for 14 of 15 loci ($P = 0.00003$)

Table 2. Average sample size (number of alleles sampled, $2N$), average number of alleles (A), average expected heterozygosity (H_e), average equilibrium heterozygosity (H_{eq}) and probability of heterozygosity deficiency (one-tailed Wilcox signed-ranks test) in weathervane scallops.

	Allozymes[a]					Microsatellites[b]				
Location	$2N$	A	H_e	H_{eq}	P	$2N$	A	H_e	H_{eq}	P(D)[c]
ADAK						229.6	16.87	0.740	0.852	0.0011
BERS	399.7	2.11	0.078	0.125	0.0047	363.3	17.93	0.732	0.853	0.0013
SHUM						344.1	17.53	0.735	0.847	0.0004
SHEL						377.3	18.53	0.725	0.863	0.0008
NEKO						369.7	18.13	0.738	0.810	0.0008
KAMI						337.1	17.40	0.735	0.855	0.0005
KAYA						376.0	18.53	0.731	0.861	0.0011
YAKU	399.8	2.34	0.081	0.151	0.0004	367.2	18.53	0.735	0.858	0.0017

[a]Includes data for all 35 loci.
[b]Includes data for 15 loci.
[c]Significance of tests for heterozygosity deficiency.

Table 3. Summary statistics for 15 microsatellite loci in weathervane scallops.

Locus	Number of alleles	P(diff)	P(D)	Estimated null allele frequency
PcauA008	21	<0.0000%5[†]	<0.00005	0.3413
PcauA101	27	0.0068	0.0405	
PcauA208	22	0.0885	<0.00005	0.0435
PcauA213	13	0.2144	<0.00005	0.0517
PcauA218	13	0.3414	0.6585	
PcauB110	40	0.8652	0.1975	
PcauB111	21	0.2121	<0.00005	0.0094
PcauB113	9	0.2485	0.5580	
PcauB211	19	0.0042[†]	<0.00005	0.1264
PcauB216	11	0.8469	0.0130	
PcauC001	57	0.3702	<0.00005	0.0128
PcauC205	12	0.3400	0.3065	
PcauD008	21	0.1961	<0.00005	0.0568
PcauD011	19	0.7715	0.2750	
PcauD101	49	0.4174	<0.00005	0.0211

Note: P(diff), probability of homogeneity among populations estimated by randomization of genotypes, not assuming HWE within samples; P(D), significance of test for heterozygote deficiency.

[†]Not significant after adjusting allele frequency estimates to account for the presence of null alleles. Null allele frequencies were estimated using *ML-NullFreq* for loci with significant heterozygote deficiencies.

in seven samples, and 13 of 15 loci ($P = 0.0026$) in one sample, also consistent with an historical population expansion. In contrast, the interlocus g-test, less sensitive to recent expansions than the k test, failed to detect population expansion in any sample (g ranged from 1.4–2.5, compared with a significance rejection value of ≤ 0.22).

Nuclear SNPs

Elfstrom et al. (2005*a*) found no sequence variation for troponin C-long (AY704167, 1129 bp) and 18S rRNA (AY704169, 792 bp) in 60 individuals from two widely separated populations (BERS and YAKU). In contrast, sequences of ODH (AY704172, 720 bp) and CaM (AY769248, 491 bp) contained nucleotide polymorphisms (two and six, respectively), three of which were used here (Appendix B). Each of the eight samples and the pooled data fit HWE. Exact R × C tests of allele frequencies for both loci showed no significant allele-frequency heterogeneity among samples.

Mitochondrial DNA

Haplotypes were constructed with data from nine mitochondrial SNPs in 1424 scallops, yielding 16 haplotypes in eight samples (Table 4). Haplotype diversity among samples ranged from $h = 0.760$ to 0.809, and was 0.781 in the pooled sample (Table 5). Nucleotide diversity was $\Theta_\pi = 0.0005$ in all samples and in the pooled sample. Values of Fu's F_S and Tajima's D were generally negative, but none was significant. (However, in a mitochondrial sequence data pilot set of 25 individuals from ADAK and BERS containing 14 variable sites and 11 haplotypes, Fu's F_S was –4.19 ($P = 0.0068$).) Five private haplotypes appeared in four samples (ADAK, SHUM, SHEL, KAMI). Haplotype frequencies were not significantly different among samples (exact R × C, $P = 0.53$), nor were the frequencies of individual mtDNA SNPs. Estimates of conventional F_{ST} between samples ranged from 0.0 to 0.011. Global differentiation ($F_{ST} = 0.0008$) was not significant. An AMOVA indicated that 0.17% of the variation was due to differences between populations in the Bering Sea and Gulf of Alaska, and that 0.02% was due to differences among samples within these two groups (Table 6). Most of the variation (99.81%) was distributed among individuals within samples. None of the corresponding Φ-statistics was significant. The correlation between F_{ST} and distance between samples was weak, but nearly significant ($P = 0.053$). Coalescence estimates of gene flow ranged from 2.0 (ADAK to SHUM) to 11.0 (SHEL to KAYA) individuals per generation and did not show a geographic pattern (data not shown).

The median-joining network showed a shallow genealogy with haplotypes connected by one mutational step (Fig. 2). Haplotype mismatch distributions were unimodal in individual samples and in the pooled sample (raggedness statistics < 0.058; Table 7; Fig. 3). All the sample mismatch distributions fit a model of population expansion. The pooled sample had a mean mismatch $\pi = 1.56$ and a $\tau = 1.61$. With mutation calibrations of 10% and 2% between lineages, the value of τ in the pooled sample yielded times since population expansion of about 5 and 27.7 ka, respectively. Estimates of $\Theta_0 = N_0 2\mu$ were 0.0 in five, largely western,

Table 4. Composite mtDNA haplotype frequencies in weathervane scallops.

Haplotype	ADAK	BERS	SHUM	SHEL	NEKO	KAMI	KAYA	YAKU	Total
1 CAAGAAGAG	40	69	57	60	54	57	65	53	455
2 CAAGAAGGG	27	41	48	51	47	46	57	52	369
3 TATGAAGAG	28	26	36	32	46	32	26	35	261
4 CATGAAGAG	27	16	21	15	18	22	23	20	162
5 CAAAGAGAG	4	5	6	9	5	8	6	11	54
6 CGTGAAGAG	5	10	5	8	3	7	7	3	48
7 CAAGATGAG	5	4	4	3	3	6	3	4	32
8 CAAGAATAG	2	4	5	2	2	1	2	1	19
9 CATGAAGAA	1	1	1			1	2	3	9
10 CAAGAAGAA			1		1	1	1	2	6
11 CAAGGAGAG					3	1			4
12 CATGGAGAG						1			1
13 CAAGAATGG				1					1
14 CAAAAAGGG			1						1
15 CAAAGAGGG	1								1
16 CAAAGATAG	1								1
TOTAL	141	176	187	187	182	180	187	184	1424

Note: Nine mtDNA polymorphisms in three amplicons were assayed to form the composite haplotype: cytochrome oxidase subunit I (GenBank AY704170:51), a noncoding region (AY704171:56, 140, 193, 197, 891, 999 and 1045), and ATPase 6 (AY704168:1083).

samples (ADAK, BERS, SHUM, SHELL, KAMI), and between 0.011 and 0.088 in three samples from the central and eastern Gulf of Alaska (NEKO, KAYA, YAKU). These latter estimates yielded pre-expansion evolutionary population sizes of 0.0–1390 females. Θ_1 ranged between 9.75 and 46.82 among five samples, but coalescence simulations were unsuccessful in three samples with shallow genealogies. In the pooled sample, $\Theta_0 = 0.0$ and $\Theta_1 = 28.06$, yielding estimates of post-expansion population sizes of 148 000 and 740 000 females.

Discussion

A geographic survey of genetic variability in weathervane scallop populations in Alaska with four classes of molecular markers detected almost no population structure. The levels of divergence among populations for the various markers were remarkably low, with $F_{ST} \leq 0.0011$ among allozyme loci and $F_{ST} = 0.0004$–0.0008 for microsatellite, SNP, and mtDNA loci. These low levels of differentiation and the lack of significant differences between populations do not reflect low statistical power. Large sample sizes, large number of molecular markers, and high levels of gene diversity provided ample statistical power to detect even limited spatial heterogeneity (Ryman et al. 2006). The genetic homogeneity among scallop populations extending over 2500 km may be due to high levels of gene flow mediated by planktonic larvae (Waples 1998) or, in part, due to the residual effects of a post-glacial population expansion from an ice-age refuge. We discuss the relative merits of these hypotheses and their implications for harvest management.

Historical demographic imprints on contemporary populations

Tests for mutation-drift equilibrium show genetic imprints of past population disturbances that are likely related to the Pleistocene glacial history of the northeast Pacific. One possible mechanism producing these disequilibria is contraction of weathervane populations into a small southern refuge during glacial maximum (Dettermann 1986; Mann and Peteet 1994), followed by a post-glacial expansion into northern areas. During the last glacial maximum (LGM) 18–20 thousand years ago, glaciers on the Alaska Peninsula and Kodiak Island flowed into the Gulf of Alaska, destroying inshore scallop habitats. At least eight glacial lobes jutted offshore from major fjords into the Gulf of Alaska during the ice-age cycle (110–10 thousand years ago) (Molnia 1986; Carlson 1989). Climate models indicate that the polar front and associated storm tracks shifted 10°–20° latitude southward (Kutzbach et al. 1993). The significant excess of low-frequencies alleles (heterozygosity deficiency) is consistent with the loss of genetic variation in a bottlenecked population and the subsequent accumulation of mutations that have not yet drifted to intermediate frequencies.

A post-glacial expansion could produce genetic imprints on contemporary populations that must be considered in an assessment of contemporary population structure. A progressive step-wise colonization can lead to latitudinal gradients in allele frequencies or genetic diversity (e.g., bay pipefish, Wilson 2006), which can persist only when gene flow between populations is limited. On the other hand, rapid large-scale colonizations can produce genetically homogeneous populations with allele frequencies and diversities similar to those in refuge populations (Hewitt 1996). When gene flow is limited by the dispersal biology of a species, genetic population structure may eventually appear. In the case of weathervane scallops it is uncertain whether migration-drift disequilibrium is also associated with mutation-drift disequilibrium detected with neutral markers. If scallop populations are in migration-drift disequilibrium, the genetic homogeneity observed in this study may be due, at least in part, to a post-glacial population expansion and not necessarily to high levels of gene flow.

Table 5. Mitochondrial DNA haplotypic variability and tests of neutrality in samples of weathervane scallops.

		No. of haplotypes							
Sample	N	Obs	Exp[a]	h	Θ_π	F_S	P	D_T	P
ADAK	141	11	13.2	0.809	0.0005	–2.032	0.227	–0.071	0.557
BERS	176	9	11.1	0.761	0.0005	–0.778	0.425	–0.189	0.478
SHUM	187	11	12.9	0.790	0.0005	–1.742	0.282	–0.001	0.551
SHEL	187	9	12.3	0.780	0.0005	–0.392	0.478	0.294	0.676
NEKO	182	10	11.9	0.774	0.0005	–1.081	0.368	–0.015	0.573
KAMI	180	12	12.7	0.790	0.0005	–2.513	0.195	–0.017	0.571
KAYA	187	10	11.3	0.760	0.0005	–1.136	0.362	–0.035	0.527
YAKU	184	10	12.7	0.789	0.0005	–0.734	0.445	0.194	0.642
Total	1424	16	18.0	0.781	0.0005	–0.2442	0.260	0.667	0.942

Note: N, sample size; h, haplotype diversity; Θ_π, nucleotide diversity; F_S, Fu's test for neutrality; D_T, Tajima's test for neutrality.

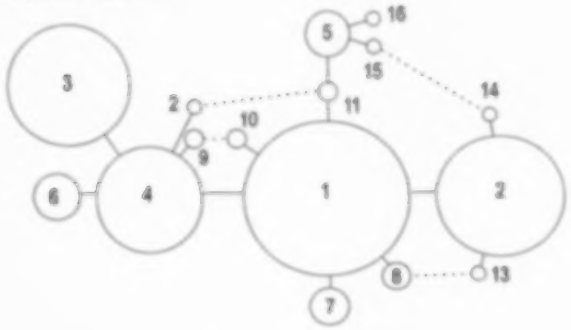
[a]Expected number of haplotypes under Ewens' (1972) neutral sampling theory.

Table 6. Analysis of molecular variance (AMOVA) of mtDNA variability among eight samples of weathervane scallops in two oceanic regions divided by the Alaska Peninsula.

Source	df	Sum of squares	Variance component	Percentage of variation	P
Between groups	1	0.736	0.00068	0.17	0.098
Among populations within groups	6	2.425	0.00008	0.02	NS
Within populations	1416	552.837	0.39042	99.81	NS

Note: F_{SC} = 0.00019; F_{ST} = 0.00192; F_{CT} = 0.00173.

Fig. 2. Median-joining network of mitochondrial DNA haplotypes in weathervane scallops. Haplotype numbers correspond to Table 4; haplotype frequencies are indicated approximately by the area of the plotting symbol.



Contemporary genetic population structure

The potential for gene flow can be assessed by considering the dispersal biology of scallops. The apparent genetic homogeneity among scallop populations may be due to high levels of gene flow promoted by dispersal of planktonic larvae. A large cyclonic gyre in the Gulf of Alaska and numerous mesoscale anti-cyclonic coastal eddies (Reed and Schumacher 1986; Hermann et al. 2002) influence patterns of larval dispersal. Coastal eddies may facilitate larval dispersal, but also may provide opportunities for self-recruiting populations by promoting larval retention (Schumacher and Stabeno 1994; Hermann et al. 2002). Evidence of long-standing isolated self-recruiting populations, however, does not appear in the distributions of molecular variants. The Alaska Current narrows and feeds the fast-moving southwestward Alaska Stream, which flows along the southern edge of the Alaska Peninsula toward the Aleutian Island Chain (Stabeno et al. 2001). The lack of a genetic break around the Alaska Peninsula may indicate that the Alaska Stream facilitates larval dispersal from the Gulf of Alaska into the Bering Sea.

Other environmental variables may also shape population structure. The patchy distribution of suitable habits, for example, may separate concentrations of adults, but this separation does not appear to limit gene flow mediated by planktonic larvae. Life-history differences between populations (e.g., growth rate, Ignell and Haynes 2000) were also thought to reflect genetic population structure, but the genetic homogeneity among populations detected here likely indicates that these differences arise on short time scales as responses to local environmental conditions.

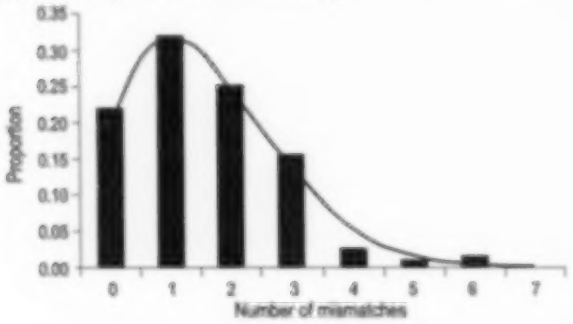
Ocean current patterns and the recruitment biology of scallops could potentially influence patterns of genetic structure. The predominant counter-clockwise currents around the Gulf of Alaska and northward flow into the Bering Sea might produce asymmetric patterns of gene flow among populations, if larval dispersal is largely limited to adjacent populations. However, Mantel's test for isolation by distance did not detect a significant relationship between genetic and geographic distances for any of the markers. Additionally, estimates of migration between populations did not show asymmetries that are characteristic of unidirectional gene flow or of source-sink relationships among populations. Sweepstakes recruitment of offspring from a few families could also produce genetic patterns (Hedgecock 1994). However, the scale of sampling does not permit an assessment of this process on the spatial scale over which this effect has been observed in other invertebrates.

Table 7. Mitochondrial DNA mismatch statistics for samples of weathervane scallops.

Sample	Mean	r	Years ($\times 10^3$)	Θ_0	Nd^a	Θ_1	N_0	Raggedness	Model P
ADAK	1.58	1.563	4.9–24.7	0	0	9999	—	0.06	0.179
BERS	1.44	1.490	4.7–23.5	0	0	46.82	147 977–739 886	0.04	0.513
SHUM	1.55	1.578	5.0–25.0	0	0	9999	—	0.05	0.192
SHEL	1.56	1.598	5.0–25.3	0	0	25.88	81 795–408 976	0.04	0.487
NEKO	1.55	1.732	5.5–27.4	0.011	35–1738	11.85	37 453–187 263	0.04	0.358
KAMI	1.55	1.578	5.0–25.0	0	0	9999	—	0.05	0.207
KAYA	1.53	1.555	4.9–24.6	0.088	278–1391	9.75	30 815–154 077	0.04	0.641
YAKU	1.69	1.754	5.5–27.7	0.063	199–996	10.57	33 407–167 035	0.04	0.527
Total	1.56	1.613	5.0–25.5	0.000	0	28.06	88 685–443 426	0.04	0.354

[a]Female evolutionary population size. Intervals defined by 2% and 10% sequence divergence per million years.

Fig. 3. Sequence mismatch distribution of mitochondrial DNA haplotypes from a pooled sample (N = 1424) of weathervane scallops. Bars indicate observed mismatches and the solid line indicates the expected distribution for a population that has experienced a recent population expansion or selective sweep.



Assessments of molecular stock structure in other species of scallops are variable. For example, a study of microsatellites in the ecologically similar scallop *Placopecten magellanicus* in the northwest Atlantic showed regional differences (F_{ST} = 0.012), but no differences among populations within regions (F_{ST} = –0.001) (Kenchington et al. 2006). The genetic breaks between regional groups largely coincided with barrier currents that apparently isolate populations on a large scale and produce genetic differences. In the European scallop *Pecten maximus*, populations were largely genetically homogeneous over an area including France, England, Ireland, and Scotland. Divergence among populations was F_{ST} = 0.005 for mtDNA (Wilding et al. 1997) and F_{ST} = 0.020 for allozymes (Wilding et al. 1998). However, in another study of this species, significant mtDNA haplotype frequency differences were detected among five inshore samples from the Isle of Man (Heipel et al. 1999).

In contrast to the genetic homogeneity in weathervane scallops, the Japanese scallop *Mizuhopecten yessoensis* shows genetic heterogeneity on two geographic scales in the northwest Pacific. On a small scale, a significant difference was detected with non-coding mtDNA sequences among populations around Hokkaido Island and a nearby population in Mutsu Bay on Honshu Island (F_{ST} = 0.155) (Nagashima et al. 2005). However, populations were generally homogeneous over large distances along the Russian coast (>1300 km) (Dolganov and Pudovkin 1998) and around Hokkaido Island (Nagashima et al. 2005). On a larger spatial scale, allozyme markers showed a deep partition among populations along the Russian coast and southern Sakhalin Island and populations extending from Pacific Japan, to Pacific Sakhalin, to the Kuril Islands (Dolganov and Pudovkin 1998). The analysis of mtDNA in a more limited set of samples confirms this partition between Japanese and Russian populations (Wilbur et al. 1997).

The results of these studies together indicate that the predominant influence on genetic population structure in scallops appears to be larval dispersal in currents. Gene flow among populations, however, is likely to be unequal among populations, because of the complexities of ocean currents and because of isolation by distance. The isolating effects of local adaptation, peninsulas, frontal currents, and ocean

gyres are expected to produce genetically differentiated populations, at least at migration-drift equilibrium. It is also possible that unsampled deep-water populations provide connectivity among coastal populations, resulting in sufficient gene flow to ensure genetic homogeneity.

Genetic homogeneity as measured with neutral molecular markers does not necessarily preclude the existence of population structure. Life history differences among populations reflecting local adaptation are not always accompanied by allele-frequency differences at neutral loci (Waples and Gaggiotti 2006). For example, *P. maximus* shows clear differences in reproductive biology among populations, but no allele-frequencies differences at eight polymorphic allozyme loci across a wide geographic range (Beaumont et al. 1993). In another example for this species, PCR-RFLP analyses of mtDNA did not detect differences between Norwegian populations with different reproductive cycles (Ridgway and Dahle 2000). Even though genetically unique populations of weathervane scallops were not detected by the presumably neutral markers in this study, some populations nevertheless may be adapted to local environmental conditions (Conover et al. 2006). This uncertainty supports a precautionary approach to the management of scallop populations (Shirley and Kruse 1995; Kruse et al. 2005) and precludes the transport of cultured spat into or across larval drift zones in Alaska (Gaffney and Allen 1992).

In conclusion, the results of this study show that both the climatic history of the northeastern Pacific and patterns of gene flow have sculpted the genetic population structure of weathervane scallops, as viewed with neutral molecular markers. While a rapid post-glacial expansion may have contributed to genetic homogeneity among colonizing populations, subsequent patterns of gene flow between populations are expected to be the predominant influence shaping contemporary population structure. Other species of scallops with populations in previously glaciated areas show significant levels of genetic structure, indicating that sufficient time has elapsed since the last glacial maximum for scallop populations to approach migration-drift equilibrium. We conclude that the genetic homogeneity among populations of weathervane scallops reflects high levels of gene flow and not the imprint of demographic history. This is a reasonable conclusion, given the fast-moving currents in the northeastern Pacific. In any case, homogeneity of neutral molecular marker frequencies does not mean that adaptive differences among populations are absent. Populations may still be ecologically independent of one another and thus require a "cautionary approach" of population-by-population management based on ecological and recruitment patterns, rather than on genetic data (Conover et al. 2006). Under these circumstances, the use of oceanographically based management districts and putative larval drift zones remains the best approach to sustaining scallop harvests.

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Appendix A and Appendix B appear on the following pages.

Appendix A

Table A1. Allozyme allelic frequencies in samples of *Patinopecten caurinus* from six localities.

Allele	BERS	SHUM	SHEL	NEKO	KAYA	YAKU	Pooled
Ak							
1	0.659	0.666	0.666	0.629	0.638	0.634	0.648
2	0.320	0.330	0.324	0.368	0.357	0.361	0.344
3	0.005	0.000	0.000	0.000	0.000	0.000	0.001
4	0.008	0.002	0.002	0.000	0.002	0.002	0.003
5	0.007	0.002	0.008	0.003	0.003	0.002	0.004
6	0.000	0.000	0.000	0.000	0.000	0.002	0.000
N	298	229	299	299	300	299	1724
F	0.102	–0.041	–0.124	–0.053	–0.059	0.079	–0.014
Gpi							
1	0.560	0.575	0.545	0.503	0.565	0.532	0.546
2	0.360	0.323	0.365	0.400	0.358	0.383	0.366
3	0.042	0.021	0.050	0.053	0.033	0.045	0.042
4	0.000	0.002	0.000	0.000	0.000	0.000	0.000
5	0.002	0.004	0.000	0.002	0.000	0.000	0.001
6	0.000	0.000	0.003	0.005	0.002	0.000	0.002
7	0.008	0.019	0.005	0.015	0.007	0.020	0.012
9	0.027	0.051	0.032	0.020	0.035	0.017	0.029
10	0.002	0.000	0.000	0.000	0.000	0.002	0.001
11	0.000	0.000	0.000	0.000	0.000	0.002	0.000
12	0.000	0.004	0.000	0.002	0.000	0.000	0.001
N	300	234	300	300	300	299	1733
F	0.052	0.014	0.066	–0.004	–0.101	0.018	0.008
Pep-1							
1	0.809	0.812	0.820	0.790	0.818	0.781	0.805
2	0.153	0.109	0.138	0.153	0.137	0.159	0.143
3	0.034	0.049	0.032	0.040	0.035	0.038	0.038
4	0.000	0.002	0.003	0.007	0.002	0.003	0.003
5	0.003	0.026	0.007	0.008	0.007	0.018	0.011
6	0.002	0.000	0.000	0.002	0.000	0.000	0.001
8	0.000	0.002	0.000	0.000	0.002	0.000	0.001
N	298	234	300	300	300	299	1731
F	0.041	0.084	0.080	–0.015	0.024	0.044	0.041
Pep-3							
1	0.863	0.868	0.886	0.863	0.858	0.857	0.866
2	0.085	0.081	0.092	0.102	0.078	0.105	0.091
3	0.028	0.019	0.007	0.012	0.020	0.017	0.017
4	0.005	0.009	0.007	0.007	0.003	0.007	0.006
5	0.007	0.015	0.007	0.003	0.013	0.007	0.008
6	0.000	0.000	0.000	0.002	0.002	0.002	0.001
7	0.012	0.009	0.000	0.012	0.020	0.007	0.010
8	0.000	0.000	0.000	0.000	0.003	0.000	0.001
11	0.000	0.000	0.000	0.000	0.002	0.000	0.000
12	0.000	0.000	0.002	0.000	0.000	0.000	0.001
N	300	234	300	300	300	300	1733
f	0.001	0.023	0.029	0.004	0.039	0.046	0.024
Pep-4							
1	0.258	0.234	0.240	0.258	0.217	0.254	0.244
2	0.223	0.242	0.238	0.261	0.227	0.226	0.236
3	0.077	0.079	0. 095	0.102	0.075	0.110	0.090
4	0.065	0.052	0.070	0.035	0.077	0.079	0.063
5	0.138	0.135	0.105	0.117	0.115	0.117	0.121
6	0.235	0.251	0.247	0.222	0.281	0.206	0.240

Table A1 (*concluded*).

Allele	BERS	SHUM	SHEL	NEKO	KAYA	YAKU	Pooled
7	0.000	0.000	0.003	0.000	0.000	0.000	0.001
8	0.000	0.000	0.000	0.002	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000	0.002	0.000
10	0.003	0.000	0.002	0.002	0.003	0.007	0.003
11	0.000	0.006	0.000	0.000	0.003	0.000	0.001
12	0.000	0.000	0.000	0.002	0.000	0.000	0.000
N	300	233	300	299	299	299	1730
f	0.025	–0.017	–0.032	0.033	0.037	–0.014	0.007
Pgm-2							
1	0.886	0.878	0.874	0.875	0.905	0.886	0.884
2	0.029	0.030	0.037	0.033	0.027	0.029	0.031
3	0.052	0.043	0.049	0.055	0.047	0.052	0.050
4	0.010	0.017	0.013	0.012	0.010	0.010	0.012
5	0.017	0.011	0.012	0.018	0.003	0.017	0.013
6	0.007	0.009	0.013	0.003	0.005	0.007	0.007
7	0.000	0.002	0.002	0.003	0.000	0.000	0.001
8	0.000	0.000	0.000	0.000	0.003	0.000	0.001
9	0.000	0.004	0.000	0.000	0.000	0.000	0.001
10	0.000	0.006	0.000	0.000	0.000	0.000	0.001
N	298	234	297	300	300	298	1727
f	0.048	0.054	0.060	0.001	0.028	0.048	0.039*

Note: *f*, Weir and Cockerham's F_{is} estimate.

Appendix B

Table B1. Nuclear SNP genotypic frequencies in *Patinopecten caurinus* populations.

ODH189				
Location	AA	AG	GG	*N*
ADAK	3	12	121	136
BERS		17	166	183
SHUM	1	22	161	184
SHEL		18	167	185
NEKO	2	13	173	188
KAMI	2	24	164	190
KAYA		16	166	182
YAKU		24	167	191
Total	8	146	1285	1439

CaM								
Location	AA	AB	AC	BB	BC	CC	AD	*N*
ADAK	104	18	17		1			140
BERS	132	38	18		1			189
SHUM	133	30	23		3	1	1	191
SHEL	130	34	18	1	5	1		189
NEKO	126	33	26	2	2	2		191
KAMI	136	27	23	2	1	1		190
KAYA	119	38	28	2	1	1		189
YAKU	130	31	23	4	1	1		190
Total	1010	249	176	11	15	7	1	1469

Note: For the CaM amplicon, four haplotypes for were inferred from SNPs 282 and 386: A (C/T), B (T/C), C (T/T), and D (C/C).

Factors influencing the relative fitness of hatchery and wild spring Chinook salmon (*Oncorhynchus tshawytscha*) in the Wenatchee River, Washington, USA

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Abstract: Understanding the relative fitness of naturally spawning hatchery fish compared with wild fish has become an important issue in the management and conservation of salmonids. We used a DNA-based parentage analysis to measure the relative reproductive success of hatchery- and natural-origin spring Chinook salmon (*Oncorhynchus tshawytscha*) in the natural environment. Size and age had a large influence on male fitness, with larger and older males producing more offspring than smaller or younger individuals. Size had a significant effect on female fitness, but the effect was smaller than on male fitness. For both sexes, run time had a smaller but still significant effect on fitness, with earlier returning fish favored. Spawning location within the river had a significant effect on fitness for both sexes. Hatchery-origin fish produced about half the juvenile progeny per parent when spawning naturally than did natural-origin fish. Hatchery fish tended to be younger and return to lower areas of the watershed than wild fish, which explained some of their lower fitness.

Résumé : Pour la gestion et la conservation des salmonidés, il est devenu important de comprendre la fitness relative des poissons d'élevage qui frayent naturellement par rapport à celle des poissons sauvages. Nous avons utilisé une analyse de filiation basée sur l'ADN pour mesurer le succès reproductif relatif de saumons chinook (*Oncorhynchus tshawytscha*) du printemps provenant d'élevages ou d'origine naturelle dans le milieu naturel. La taille et l'âge ont une forte influence sur la fitness des mâles, car les mâles plus grands et plus âgés produisent plus de descendants que les individus plus petits ou plus jeunes. La taille a un effet significatif sur la fitness des femelles, mais cet effet est moins important que sur la fitness des mâles. Chez les deux sexes, le moment de la montaison a un effet petit mais néanmoins significatif sur la fitness et les poissons qui reviennent tôt sont favorisés. Le site de fraie dans la rivière a un effet significatif sur la fitness des deux sexes. Lorsqu'ils frayent naturellement, les poissons d'élevage produisent environ la moitié de la descendance par parent que ne le font les poissons d'origine naturelle. Par comparaison aux poissons sauvages, les poissons d'élevage ont tendance à être plus jeunes et ils retournent à des sites plus en aval dans le bassin versant, ce qui explique en partie leur fitness réduite.

[Traduit par la Réduction]

Introduction

Artificial propagation is a commonly used tool to conserve a wide variety of threatened species (Mallinson 1995). Hatchery propagation, in which fish are bred and reared for part of their lives in captivity before being released into the wild, is widely used to supplement wild salmon populations (Naish et al. 2007). For example, over 4 billion anadromous juvenile salmon are released annually into the North Pacific Ocean from hatcheries in North America and Asia (Beamish et al. 1997). Similar hatchery programs and large-scale closed-pen fish farming operations exist for Atlantic salmon (*Salmo salar*) in the North Atlantic Ocean and Baltic Sea. Hatcheries are increasingly intended to contribute to conserving natural salmonid populations, as well as to produce fish to mitigate for lost harvest opportunities (National Research Council 1996). In particular, supplementation projects, in which natural spawning by hatchery fish is intended to augment a natural population's abundance, have become common throughout the Pacific Northwest (Naish et al. 2007) and Europe (Fleming et al. 2000).

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A key biological uncertainty about the effects of hatchery production on natural populations is the degree to which hatchery-produced fish can reproduce in the natural environment (Reisenbichler and McIntyre 1977; Ford 2002; Araki et al. 2008). Evaluating relative reproductive success is therefore critical for determining if the considerable investment society has made in hatchery supplementation is actually contributing to the recovery of salmon populations (Mobrand et al. 2005). Accurately measuring the biological causes of variance in reproductive success is important not only for determining the benefits of conservation hatcheries, but also for evaluating the risks from fish that stray from "production" type hatcheries. The presence of large numbers of hatchery fish on spawning grounds can obscure the status of natural populations because their reproductive success is unknown (McClure et al. 2003) and may lead to reduced short- and long-term natural productivity because of genetic deterioration of the natural population as a result of interbreeding between naturally produced fish and hatchery fish (Lynch and O'Hely 2001; Ford 2002). By quantifying the reproductive success of hatchery fish relative to that of fish from the natural population, the viability of natural populations receiving hatchery fish can be more accurately evaluated.

Even when the relative reproductive success of hatchery-produced fish is quantified, the causes of fitness differences between hatchery and wild fish often remain unknown. Conceptually, there could be a wide variety of reasons why hatchery fish might have lower fitness than wild fish spawning in the same stream (Araki et al. 2008). Even in cases where hatchery fish have low fitness, the conservation implications are likely to vary depending on why the hatchery fish are less fit. For example, if reduced fitness is largely due to environmental effects such as release location, this would probably lead to fewer conservation concerns than if fitness reductions were due to genetic differences in behavior or physiology.

In a recent review, Araki et al. (2008) concluded that hatchery-produced steelhead (i.e., sea-run rainbow trout, *Oncorhynchus mykiss*) generally have lower reproductive success in the natural environment than wild steelhead. In contrast, they noted that few data are available on the relative reproductive success of hatchery and wild Chinook salmon (*Oncorhynchus tshawytscha*) despite the extensive use of hatchery supplementation for this species (Independent Scientific Advisory Board 2003, 2005; Araki et al. 2008). Recently, several papers have reported on studies of hatchery Chinook salmon breeding success in laboratory or semi-natural environments (Fritts et al. 2007; Pearsons et al. 2007; Schroder et al. 2008), but there are no published studies of the relative fitness of this species in the wild.

In this study, we assess the relative reproductive success of naturally spawning hatchery- and natural-origin spring run Chinook salmon in the Wenatchee River, Washington, USA, by employing a genetic pedigree analysis and determine the degree to which differences in reproductive success between hatchery and natural Chinook salmon can be explained by biological characteristics such as run timing, morphology, and spawning location.

Materials and methods

Study population

Our study population consists of the spring run Chinook salmon that spawn in the Wenatchee River, Washington (Fig. 1). The population is listed as "endangered" under the Endangered Species Act (Federal Register 70:37160). Starting in 1989, hatchery supplementation has been used in an attempt to increase population abundance, and in recent years the hatchery program has produced >50% of the individuals in the population that spawn naturally. The hatchery program's focus is primarily on the Chiwawa River, a major tributary of the Wenatchee River. Wild and hatchery-origin broodstock for the supplementation program are collected at a weir in the Chiwawa River, and the offspring of those fish are released back into the Chiwawa River as yearlings. Although juvenile fish are released only in the Chiwawa River, as adults they return to spawn in all of the major spawning areas throughout the watershed (Murdoch et al. 2008).

The population exhibits a "stream-type" life-history pattern (Healey 1991) in which adults return to fresh water in the spring several months prior to spawning, and juveniles migrate to the ocean during the spring 1 year following their emergence from the gravel (Healey 1983). In wild populations of Chinook salmon in the Columbia River, including the Wenatchee River, most anadromous males become sexually mature between ages three to five, and most females become mature at ages four or five (Myers et al. 1998). Another characteristic of stream-type Chinook salmon is that some male fish mature at 1 or 2 years of age without migrating to the sea (Rich 1920; Burck 1967; Mullan et al. 1992), but little is known about the reproductive success of these early maturing males.

Adult and juvenile trapping and sampling

In 2004 and 2005, beginning in early April and ending in early August, essentially all migrating spring Chinook salmon were trapped and sampled (scales, caudal fin clip) at Tumwater Dam, located at river kilometre (rkm) 43.7 on the Wenatchee River (Fig. 1, Table 1). Tumwater Dam is located below all of the major spring Chinook salmon spawning areas in the watershed, so we obtained samples from essentially all of the potential breeders, with the possible exception of those mature male parr that never migrated below Tumwater Dam and a very small number of adults that migrated after the trapping period ended. Biological data were collected from all adult salmon sampled (Table 1). Each fish was identified to gender, scanned for passive integrated transponder (PIT) tags (fish without a PIT tag had one inserted during sampling) and coded wire tags and the presence or absence of the adipose fin (indicating hatchery or wild origin; all hatchery fish released in the Wenatchee watershed have a clipped adipose fin). Fork and post orbital to hypural plate length were measured to the nearest centimetre and weight to the nearest 0.01 kg. Subsequent identification of PIT-tagged carcasses recovered on the spawning grounds permitted the comparison of carcass recovery distributions of individual hatchery- and naturally produced fish. Spawning location was based upon location of carcass recovery and linked back to fish identity at Tumwater Dam

Fig. 1. Map of the study area in Washington, USA. Major spring Chinook salmon spawning tributaries include the Chiwawa River, Nason Creek, White River, and Little Wenatchee River. Parents were sampled at Tumwater Dam, and progeny were sampled at White Trap, Nason Trap, Chiwawa Hatchery, and Lower Wenatchee Trap.

Table 1. Summary of 2004 and 2005 adult and juvenile Chinook salmon samples, stratified by year, origin, and life stage.

	Adults			Yearling juveniles			Subyearling juveniles	
Year	Origin	Males	Females	C	N	LW	C	N
2004	Hatchery	1502	270					
	Wild	437	376	744	203	647	576	447
	Unknown	18	13					
2005	Hatchery	1573	1724					
	Wild	235	238			889		
	Unknown	15	17					

Note: Trap locations are as follows: C, Chiwawa River; N, Nason Creek; LW, Lower Wenatchee River.

by PIT tag information. Spawning ground surveys of all potential spawning habitat were conducted twice a week throughout the entire spawning season (Murdoch et al. 2008). Carcass recovery location of each PIT-tagged fish was recorded using handheld GPS devices and converted to river kilometre using ArcView 9.2 (ESRI, Redlands, California).

Juvenile Chinook salmon samples (caudal fin clip) were taken from fish collected in rotary screw traps located on the lower Wenatchee River (rkm 9.6), Chiwawa River (rkm 1.0), and Nason Creek (rkm 0.8) (Fig. 1, Table 1). All rotary traps were located downstream from the majority of spawning habitat in each stream. The primary collection location was the lower Wenatchee River, which is below all spawning areas and operated from early February through August, although most yearling smolts were captured prior to 1 July. Depending on river discharge levels, one or two screw traps (1.5 m diameter) were operated, and trap efficiency ranged between 1% and 3%. Because of spring runoff, traps operated during 83% and 92% of the trapping period in 2006 and 2007, respectively. At tributary trap locations, yearling

(age 1) juveniles were sampled approximately daily from early March to late June. In addition, separate samples of subyearlings (age 0) juveniles produced by the 2004 spawners were collected weekly from late September to early November on the Chiwawa River and Nason Creek in 2005 (Table 1).

Microsatellite genotyping

Genomic DNA was extracted from fin clips according to the method of LaHood et al. (2008). All individuals were genotyped at 11 microsatellite loci: Ots3 (Banks et al. 1999); Ots104 (Nelson and Beacham 1999); Ots201b, Ots211, and Ots213 (Greig et al. 2003); Ots2M and Ots10M (Greig and Banks 1999); Ots519NWFSC (Naish and Park 2002); Oke4 (Olsen et al. 1998); Ogo4 (Olsen et al. 1998); and Ssa408 (Cairney et al. 2000). Microsatellite loci were amplified by polymerase chain reaction (PCR), and allele sizes were electrophoretically resolved and scored according to the method of Winans et al. (2004). For each fluorescent phosphoamidite-labeled primer set, the annealing temperature used was 48 °C (Ots3 and Ots104), 54 °C (Oke4, Ots10M, Ots213, and Ots519NWFSC), or 60 °C (Ogo4, Ots2M, Ots201b, Ots211, and Ssa408). Genotyping error rate per locus was determined by re-amplifying and re-scoring microsatellite loci for a subset of individuals and calculating the number of alleles mis-scored over the total number of alleles observed at each locus. Error rates varied among loci and were generally ~1%.

Parentage assignment

Parentage assignment methods and results are described in detail in a previous paper (Ford and Williamson 2009). Briefly, assignment of parentage was calculated using the likelihood methods of Meagher and Thompson (1986) and Gerber et al. (2000) as implemented in the program FAMOZ (Gerber et al. 2003). Individuals with missing data at more than one locus were excluded from the analysis. In addition, a small number (<2% of the samples) of summer run Chinook salmon, which are genetically distinct from the spring run population (Utter et al. 1995; Schwenke et al. 2006), were excluded from the analysis on the basis of their genotypes. Each individual in a sample of progeny was tested against all potential pairs of parents, and a log of odds score was calculated for each potential pair of parents and offspring as the log of the ratio of the probability of a parent pair – offspring relationship compared with the probability they were drawn randomly from the population. The analysis assumed a per locus genotyping error rate of 0.1%, which was lower than our estimated error rate. In evaluating simulated data, however, it produced more accurate results than an error rate of either 0% or 1.5%, similar to what has been reported previously (Sancristobal and Chevalet 1997; Gerber et al. 2000).

Ford and Williamson (2009) discovered a pattern of biased assignment failure, such that progeny of hatchery fish in this population were less likely to be assigned to the correct pair of parents than progeny of wild fish, because of higher levels of relatedness among the hatchery-origin fish. In situations where multiple parent pairs are compatible with some offspring, fractional assignment methods provide a statistically robust way to estimate selection gradients (e.g., Morgan and Conner 2001; Nielsen et al. 2001) and fitness differences between groups (Ford and Williamson 2009). In particular, Ford and Williamson (2009) found that both fractional assignment and assignment to the most likely parent pair without use of a statistical threshold for assignment produced unbiased estimates of relative fitness. For the fractional assignments, only the 20 most likely parent pairs for each offspring were included in the analysis rather than every possible combination of parents. The rationale for this was to make the calculations computationally tractable. The specific value of 20 was chosen because the likelihood value of the nth most likely parent pair declines rapidly with increasing n, and likelihoods were essentially zero by $n = 20$. As a point of comparison for the fractional assignment results, we also conducted analyses based solely on the single most likely pair of parents for each progeny (i.e., $n = 1$).

Relative fitness and selection analysis

The fitness of an individual spawner was defined as either the number of offspring assigned to that spawner (for the analyses using the single most likely pairs of parents) or the number of fractionally assigned offspring (for the analysis using the fractional assignments). For the fractional assignments only, the initial fitness values were standardized by the mean fitness values within sexes and years.

In addition to simply comparing the mean fitness estimates of wild and hatchery fish within sexes and years, we also simultaneously evaluated the effects of the following traits on fitness: weight, run time (day of year), age, origin (hatchery or natural), and spawning location. To facilitate comparisons among sexes, traits, and years and to identify which covariates had the largest effects on fitness, traits were standardized within each sex and year by subtracting the mean and dividing by the standard deviation. Weight was cube-root-transformed prior to standardization such that it was a more linear function of length, and run time was converted to ordinal days for ease of analysis and to permit comparison between years. For the relative fitness estimates based on the fractional assignments, linear regression models were used to test the significance of each covariate following Lande and Arnold (1983). For the ith parent, relative fitness can be written as

$$Y_i = B_0 + \sum B_j \cdot \text{trait}_j + \varepsilon_i$$

where $\varepsilon_i \sim \text{Normal}(0,\sigma)$, and traits refer to origin, age, weight, run timing, and (for some models) spawning location. For calculation of p values in the linear models only, the relative fitness values were transformed by raising to the 0.2 power to improve normality. All effect estimates and comparisons between hatchery and wild fitness, however, are reported using the untransformed relative fitness values (Lande and Arnold 1983). Because the relative fitness estimates based on the single most likely assignments are discrete variables, we used generalized linear models (GLMs) with a Poisson error distribution ("glm" in the statistical package R with log-link and treatment contrasts). This framework assumes that the offspring for the ith parent have an approximately Poisson distribution (u_i). Covariates are used to predict the natural log of the mean:

Table 2. Summary of genetic variation in the 2004 and 2005 hatchery and wild parents and their offspring.

			Parents (H_O/H_E)				Offspring (H_O/H_E)	
			2004		2005		2004	2005
Locus	Alleles	Exclusion probability	Hatchery (n = 1771)	Wild (n = 812)	Hatchery (n = 3294)	Wild (n = 473)	(n = 2524)	(n = 873)
Ogo4	18	0.824	0.82/0.81	0.78/0.82	0.82/0.83	0.84/0.82	0.79/0.81	0.81/0.83
Ots10M	7	0.361	0.51/0.54	0.53/0.55	0.56/0.53	0.56/0.55	0.54/0.54	0.53/0.55
Ots211	44	0.975	0.94/0.94	0.95/0.94	0.95/0.94	0.95/0.94	0.91/0.94	0.94/0.94
Ots213	36	0.952	0.94/0.91	0.90/0.91	0.93/0.90	0.91/0.91	0.89/0.90	0.91/0.91
Ots2M	16	0.392	0.56/0.54	0.53/0.56	0.58/0.55	0.52/0.55	0.53/0.55	0.57/0.58
Oke4	11	0.552	0.62/0.63	0.55/0.61	0.61/0.64	0.62/0.62	0.57/0.60	0.64/0.64
Ots104	67	0.984	0.96/0.95	0.93/0.95	0.95/0.95	0.93/0.95	0.94/0.95	0.94/0.95
Ots201b	36	0.970	0.95/0.93	0.92/0.93	0.93/0.93	0.92/0.93	0.91/0.93	0.87/0.94
Ots3	12	0.550	0.66/0.64	0.54/0.55	0.64/0.61	0.58/0.58	0.58/0.58	0.59/0.62
Ots519	12	0.614	0.66/0.70	0.72/0.70	0.65/0.67	0.66/0.68	0.71/0.70	0.68/0.69
Ssa408	27	0.938	0.86/0.89	0.87/0.91	0.88/0.90	0.86/0.89	0.86/0.90	0.88/0.91
Average	26	0.737	0.77/0.77	0.75/0.77	0.77/0.77	0.76/0.77	0.75/0.76	0.76/0.78
Cumulative	286	>0.999999						

Note: "Alleles" refers to the total number alleles in the combined samples. Exclusion probability was calculated using the formulas reported in Jamieson and Taylor (1997) as implemented in the FAMOZ program. It is the theoretical probability that a random pair of individuals drawn from a randomly mating population with the same allele frequencies as the observed parental population would be excluded as the parents of a randomly drawn offspring. H_O and H_E refer to observed and expected (under random mating) heterozygosity, respectively. The table includes all samples with data at five or more loci.

$$\log(w_i) = B_0 + \sum B_j \cdot \text{trait}_j$$

There are several differences between the GLM approach and standard regression approach. With GLMs, we assume that the relationship between covariates and relative fitness is linear in log space or exponential in normal space. The identity link function may be used in some cases to force the relationship to be linear in normal space; however, most ecological data sets (including this one) result in convergence problems because the mean of the Poisson should never be allowed to be negative. A second important difference in these approaches is that the linear regression model introduces one additional parameter (resulting in slightly more flexibility) because it explicitly estimates a residual error parameter. Hierarchical versions of GLMs can be modified to include residual error, but because significance testing and model selection are not straightforward for these models, we did not consider them here.

Results

Genetic variation

Across the 11 loci, both hatchery-origin and wild-origin adults and their offspring had high and approximately equal levels of variation (Table 2). The overall level of genetic differentiation among the four adult origin × year combinations was low (F_{ST} = 0.0039), but exact tests of differentiation among all pairs of populations defined by origin and sample year were highly significant ($p < 0.00001$ for all comparisons). The low level of differentiation is consistent with the high rates of gene flow between the hatchery and wild subgroups expected in this supplemented population. Expected two parent exclusion probabilities were very high: >0.999999 for both hatchery- and wild-origin parents. Genotypic frequencies at several loci were significantly ($p < 0.0001$) out of Hardy–Weinberg expectations, but the absolute differences between observed and expected heterozygosity at all loci were small (Table 2) and likely represent a combination of nonrandom mating and failure to detect all large alleles at these highly polymorphic loci. The parentage assignment methods used are robust to both of these phenomena.

Phenotypic differences between hatchery and wild fish

Hatchery and wild fish differed in several characteristics (Table 3). In particular, in 2004 most of the hatchery males were 2- and 3-year-olds, whereas most of the wild males were 4-year-olds. The large numbers of 2-year-old hatchery males in 2004 led to a highly male biased sex ratio for hatchery fish in that year. For some comparisons within age, sex, and year, hatchery and wild fish differed significantly in run timing and weight, but these comparisons were not consistent between years.

One notable difference between hatchery and wild fish of both sexes was carcass recovery location. Within both the Chiwawa River and Nason Creek (the two largest spawning tributaries), hatchery fish were recovered significantly lower in the watersheds than wild fish (Table 3). There were also areas, such as the Wenatchee River main stem, that had large numbers of hatchery carcass recoveries but few or no wild carcasses.

Fitness of hatchery and wild fish in the stream environment

The fitness distribution estimated from the fractional offspring assignments was highly skewed, with a mode near zero and a tail of larger fitness values (Fig. 2). Such a highly skewed fitness distribution is similar to what has

Table 3. Summary of trait variation in adult hatchery and wild spring Chinook salmon used in the parentage analysis.

		Males							Females						
		Hatchery			Wild				Hatchery			Wild			
	Age	*n*	Mean	SD	*n*	Mean	SD	*p*	*n*	Mean	SD	*n*	Mean	SD	*p*
Weight (kg)															
2004	2	626	0.12	0.11	—	—	—	—	—	—	—	—	—	—	—
	3	747	1.79	0.64	28	1.44	0.50	<0.001	3	3.27	0.46	0	—	—	—
	4	96	5.60	1.29	403	5.32	1.30	0.06	252	5.50	0.97	362	5.28	0.81	<0.001
	5	2	9.10	0.42	4	7.71	1.10	0.09	3	6.23	2.25	7	8.23	1.77	0.26
2005	2	290	0.10	0.031	—	—	—	—	—	—	—	—	—	—	—
	3	129	1.83	0.46	9	1.62	0.30	0.08	0	—	—	0	—	—	—
	4	1111	6.05	1.30	185	5.16	1.30	<0.001	1702	5.41	0.87	201	5.39	0.97	0.80
	5	6	8.10	2.20	39	9.23	1.93	0.28	8	7.23	1.41	44	7.99	1.31	0.19
Run timing (Julian days)															
2004	2	626	193.18	9.01	—	—	—	—	—	—	—	—	—	—	—
	3	747	179.61	9.21	28	178.71	12.42	0.71	3	185.30	2.89	0	—	—	—
	4	96	173.91	14.50	403	170.73	13.58	0.05	252	177.13	12.29	362	170.13	11.70	<0.001
	5	2	167.00	2.83	4	160.00	6.22	0.13	3	169.00	14.73	7	178.71	12.65	0.39
2005	2	290	191.22	12.66	—	—	—	—	—	—	—	—	—	—	—
	3	129	179.76	11.02	9	182.44	17.74	0.67	0	—	—	0	—	—	—
	4	1111	171.79	12.66	185	175.46	15.84	<0.001	1702	170.57	12.18	201	175.01	15.39	<0.001
	5	6	166.33	16.71	39	166.97	18.91	0.93	8	164.88	16.34	44	168.39	17.03	0.59
Location[a]															
Chiwawa River															
	3	25	19.35	11.54	2	20.48	3.18	0.75	0	—	—	0	—	—	—
	4	89	18.66	10.81	47	27.57	10.64	<0.001	194	20.26	11.86	63	29.54	11.20	<0.001
	5	0	—	—	3	28.77	12.93	—	0	—	—	8	28.83	8.77	—
Nason Creek															
	3	37	8.94	6.48	1	13.44	—	—	0	—	—	0	—	—	—
	4	54	6.73	5.64	48	13.60	7.09	<0.001	118	7.92	6.34	48	13.63	8.31	<0.001
	5	0	—	—	1	13.46	—	—	1	13.37	—	15	14.95	7.20	—
White River															
	3	0	—	—	0	—	—	—	0	—	—	0	—	—	—
	4	2	29.19	0.39	5	29.33	0.41	0.71	29	30.00	0.74	11	29.70	0.70	0.24
	5	0	—	—	0	—	—	—	0	—	—	0	—	—	—
Wenatchee River															
	3	3	83.61	2.71	0	—	—	—	0	—	—	0	—	—	—
	4	37	83.76	1.90	1	78.27	—	—	60	83.25	2.36	1	85.52	—	—
	5	0	—	—		—	—	—	0	—	—	1	84.27	—	—
Little Wenatchee River															
	3	0	—	—	0	—	—	—	0	—	—	0	—	—	—
	4	5	11.52	2.23	6	11.88	1.45	0.76	22	11.14	2.91	10	11.62	2.48	0.64
	5	0	—	—	0	—	—	—	0	—	—	0	—	—	—

Note: SD, standard deviation.

[a]Location refers to river kilometres from named river mouth. Data from 2004 and 2005 are combined.

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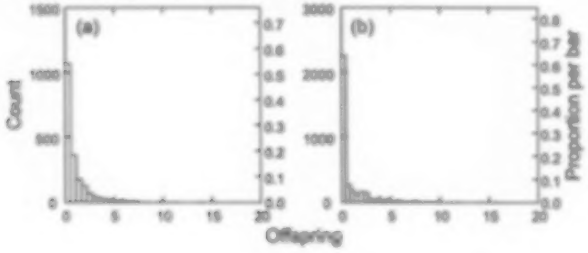
Fig. 2. Histograms of estimated fitness (fractionally assigned offspring) for 2004 (*a*) and 2005 (*b*). Both absolute estimates (counts, left-hand axis) and the proportions per histogram category (proportion per bar, right-hand axis) are shown.



been observed for other salmonid species, including coho salmon (*Oncorhynchus kisutch*; Ford et al. 2006) and steelhead (Seamons et al. 2004; Araki et al. 2007*c*). The fitness distribution based on the most likely assignments appeared very similar (data not shown).

When progeny were counted as subyearlings (2004 brood year only), we found no significant difference in progeny per parent for hatchery- and natural-origin fish if the comparisons were made within age classes, although hatchery fish produced fewer sampled progeny/parent for all age classes except age 5 females (Table 4). When progeny were counted at the yearling stage, the 4-year-old (dominant) age class hatchery fish produced significantly fewer progeny per male and female parent than did 4-year-old wild fish in both 2004 and 2005. When all age classes were combined within each sex in either 2004 or 2005, hatchery males and females produced significantly fewer yearling progeny per parent compared with their wild counterparts.

In addition to wild origin, higher weight and earlier run timing were also associated with greater fitness (Fig. 3, Table 5). The effect of weight was more important than the effect of run timing and was larger for males than for females (Table 5). Hatchery origin still had a significant negative effect on fitness, after taking into account the effects of weight, run timing, and age (Table 5). A model that used progeny counts inversely weighted by daily trapping efficiency produced essentially identical results (data not shown).

We also investigated the effect of carcass recovery location, which was measured on the spawning grounds instead of at Tumwater Dam. Only ~10% of the fish sampled at the dam were recovered as carcasses, so we combined data from both years for this analysis. Because only the Chiwawa River and Nason Creek had large numbers of hatchery and wild spawners with a range of recovery locations, we limited our analysis to these two streams. Carcass recovery location had a significant effect on fitness, with fish that spawned higher upstream producing more progeny than fish spawning downstream (Fig. 4, Table 6). For both the linear and GLM fitness models, the hatchery-origin effect was reduced (becoming less negative) when recovery location was introduced as a covariate (Table 6). For females, the hatchery coefficient in the linear model becomes nonsignificant and very close to zero when spawning location is included as a covariate, although the hatchery effect remains significant (but reduced in magnitude) in the GLM.

Table 4. Mean progeny counts based on fractional assignments, and relative fitness of hatchery and wild fish for the 2004 and 2005 spawning years.

		2004											2005									
		Subyearling						Yearling					Yearling									
		Wild			Hatchery				Wild		Hatchery			Wild			Hatchery				
Sex	Age	n^a	Mean[b]	SD	n	Mean	SD	H/W[c]	Mean	SD	Mean	SD	H/W	n	Mean	SD	n	Mean	SD	H/W
Male	2	0	—	—	636	0.22	0.50	—	—	—	0.32	0.50	—	0	—	—	290	0.20	0.50	—
	3	28	1.06	2.03	760	0.72	1.32	0.68	0.84	1.19	0.70	1.01	0.83	9	2.03	3.32	129	0.74	1.34	0.36
	4	403	2.48	3.44	96	2.00	2.50	0.81	2.50	3.12	1.38	1.49	0.55*	185	2.04	3.29	1111	1.00	2.10	0.49**
	5	4	1.15	1.40	2	0.78	1.07	0.68	4.50	7.33	0.07	0.07	0.02	39	2.05	3.32	6	1.36	2.60	0.66
	All	435	2.38	3.37	1491	0.59	1.26	0.25**	2.42	3.17	0.58	0.92	0.24**	233	2.04	3.25	1536	0.83	1.96	0.40**
Female	4	362	1.04	1.37	252	0.89	1.14	0.86	1.34	1.37	0.69	0.10	0.55**	201	1.80	2.90	1702	0.88	1.70	0.49*
	5	7	0.32	0.35	3	0.69	0.58	2.15	0.54	0.47	0.38	0.34	0.70	44	1.97	2.34	8	1.72	2.53	0.78
	All	369	1.04	1.36	271	0.91	1.17	0.88	1.23	1.36	0.68	0.96	0.55**	245	1.83	2.80	1759	0.88	1.70	0.48**

Note: Parents with data missing at >1 locus were excluded from the analysis.

[a] n refers the parental sample size for the specific origin, sex, age and year class in questions. The parental samples sizes for the 2004 subyearlings and yearlings are identical, since it the same set of parents but two different samples of offspring.

[b] Mean and SD refer to the mean and standard deviation, respectively, of the number of progeny per parent for specific origin, sex, age, and year class in question.

[c] H/W is the ratio of mean progeny number between hatchery and wild fish for specific age, sex, and year classes. Significance of differences in mean progeny number between hatchery and wild fish were calculated using a t test with bootstrapped p values to account for non-normality and are indicated with asterisks: *, $p < 0.05$; **, $p < 0.01$.

Table 8. Effects of origin, age, run timing, and weight on male and female relative fitness.

	Fractional assignment (LM)[a]				Discrete assignment (GLM)[b]			
	2004		2005		2004		2005	
Effect	Male	Female	Male	Female	Male	Female	Male	Female
Intercept	0.94 (0.12)	1.20 (0.06)	1.74 (0.13)	1.82 (0.12)	–0.19 (0.08)	0.89 (0.03)	–0.15 (0.07)	–0.29 (0.08)
Weight	0.80 (0.09)	0.18 (0.05)	0.35 (0.07)	0.12 (0.04)	0.79 (0.06)	0.20 (0.03)	0.27 (0.04)	0.09 (0.03)
Origin (hatchery effect)	–0.79 (0.15)	–0.50 (0.1)	–0.96 (0.14)	–0.94 (0.13)	–0.62 (0.09)	–0.57 (0.06)	–0.66 (0.08)	–0.70 (0.08)
Run time	–0.15 (0.05)	–0.15 (0.05)	–0.08* (0.05)	–0.18 (0.04)	–0.09 (0.03)	–0.15 (0.03)	–0.07* (0.03)	–0.15 (0.03)
Age 3 effect[c]	0.95 (0.22)	—	0.63* (0.24)	—	0.80 (0.15)	—	0.39 (0.17)	—

Note: Age 2 males were excluded and ages 4 and 5 were combined for the analysis. Results from two parentage assignment methods and fitness models are presented: fractional assignment, using models with normal error, and the single most probable parent pair using generalized linear models (GLMs) with Poisson error. Standard deviations of covariate estimates are given in parentheses; coefficients with asterisks (*) represent nonstatistically significant results ($p > 0.05$). All other coefficients are significant (at least $p < 0.05$).

[a]Coefficients of the linear model (LM) based on fractional parentage assignment: progeny = intercept + return time + effect of hatchery origin + weight + effect of age 3 (males only) + ε (normal).

[b]Coefficients of Poisson generalized linear model (GLM) based on modly likely parentage assignments: $\log(u_{progeny}) =$ intercept + return time + effect of hatchery origin + weight + effect of age 3 (males only).

[c]The age 3 effect is the effect of being age 3 (instead of ages 4 or 5), independent from the effect of weight. It is only included in the analysis of males since all females were either age 4 or 5.

Fig. 3. Relationship among age, weight, and relative fitness for the combined 2004 and 2005 data for naturally spawning males (*a*) and females (*b*). Individual relative fitness was estimated as the fractionally assigned juvenile yearling progeny count for an individual divided by the mean counts per individual within sexes and years. Individuals of ages 3, 4, and 5 are indicated by solid circles, gray squares, and open circles, respectively. Density plots of weight (kg) and fitness are illustrated on the margins for ages 3 (black), 4 (gray), and 5 (open). The density plots are scaled to reflect distributions within age classes, and heights of the plots are not proportional to the relative abundance among age classes.

Fig. 4. Relationship between female and male carcass recovery location and relative fitness for the Nason Creek females (*a*) and males (*b*) and Chiwawa River females (*c*) and males (*d*) in 2004 and 2005 combined. Individual relative fitness for wild (open squares) and hatchery (solid circles) fish was estimated as the fractionally assigned yearling juvenile count for an individual divided by the mean counts per individual within sexes. Density plots of recovery location (stream kilometre) and fitness are illustrated on the margins for hatchery (filled) and wild (open) fish. The density plots are scaled to reflect distributions within origin classes, and heights of the plots are not proportional to the relative abundance among origin classes. Dashed and solid lines illustrate the linear relationship between spawning location and the standardized progeny counts for hatchery and wild fish, respectively.

Table 6. Effects of origin, age, run timing, weight and spawning location on male and female relative fitness.

	Fractional assignment (LM)[a]				Discrete assignment (GLM)[b]			
	Male		Female		Male		Female	
Effect	Location	No location	Lococation	No location	Location	No location	Location	No location
Intercept	1.67 (0.24)	1.90 (0.24)	1.49 (0.19)	1.84 (0.20)	0.23 (0.10)	0.35 (0.10)	0.37 (0.07)	0.65 (0.06)
Weight	0.63 (0.16)	0.56 (0.16)	0.22* (0.10)	0.26 (0.11)	0.56 (0.07)	0.56 (0.07)	0.03* (0.04)	0.07 (0.04)
Origin (hatchery effect)	–0.47 (0.29)	–0.76 (0.29)	0.01* (0.22)	–0.49 (0.24)	–0.68 (0.12)	–0.79 (0.12)	–0.69 (0.10)	–0.95 (0.09)
Run time	0.19* (0.13)	0.09* (0.13)	0.10* (0.10)	–0.11* (0.11)	0.12 (0.05)	0.04* (0.05)	0.07* (0.05)	–0.03 (0.04)
Age 3 effect	0.44 (0.38)	0.30* (0.38)	—	—	0.81 (0.19)	0.75 (0.19)	—	—
Location	0.53 (0.13)	—	0.92 (0.10)	—	0.28 (0.05)	—	0.45 (0.05)	—

Note: Data are from 2004 and 2005 combined. For comparison, we included two models, one without the effect of location and one with location as a predictor. Age 2 males were excluded from the analysis and ages 4 and 5 were combined. The data used are the subset of all data for which location were available. Results from two parentage assignment methods and fitness models are presented: fractional assignment, using linear models (LM) with normal error, and the single most probable parent pair using generalized linear models (GLMs) with Poisson error. Standard deviations of covariate estimates are given in parentheses; coefficients with asterisks (*) represent nonstatistically significant results ($p > 0.05$). All other coefficients are significant (at least $p < 0.05$).

[a]Coefficients of the linear model based on fractional parentage assignment: progeny = intercept + return time + effect of hatchery origin + weight + effect of age 3 (males only) + ε (normal).

[b]Coefficients of Poisson GLM based on mostly likely parentage assignments: $\ln(\mu_{progeny}) = \text{intercept} + \text{return time} + \text{effect of hatchery origin} + \text{weight} + \text{effect of age 3 (males only)}$.

Discussion

Fitness distribution

Like previous studies of salmon fitness, the estimated offspring numbers per parent in our study were highly skewed. There are several potential causes of this skewed distribution. First, the number of offspring sampled was a small fraction of the total juvenile population, and if more progeny were sampled the fitness distribution would move to the right. Second, many of the adults in fact produced no progeny, because prespawn mortality in this population is as high as 50% (Murdoch et al. 2008). Finally, even among those fish that spawned, there appears to be a skewed distribution of progeny number, indicating a high variance in reproductive success.

Factors associated with low fitness of hatchery fish

Hatchery-origin adults in our study had less than half the mean fitness of their wild counterparts. This level of fitness reduction is similar to what has been observed in other species, particularly steelhead (e.g., Leider et al. 1990; Kostow et al. 2003; Araki et al. 2007*a*, 2007*b*). Like most steelhead hatchery programs, spring run Chinook salmon typically spend a full year rearing in the hatchery prior to release. If reduced fitness is correlated with hatchery residence time, then species that spend similar periods of time in hatchery rearing conditions might be expected to have similar reductions in fitness in the wild. However, a portion of the fitness differences we observed was explained by differences in spawning location, indicating that factors other then simply time spent in the hatchery can influence subsequent fitness.

For males, the relative fitness of hatchery fish was lower if all age classes were combined than if analyzed separately, because of the higher fraction of 3-year-old males among hatchery fish compared with wild fish. Hatchery fish have a tendency to mature at earlier ages than wild fish (Knudsen et al. 2006; Murdoch et al. 2008), but the difference is subtle and does not explain the differences in age structure we observed. Instead, these differences were due mostly to an increase in hatchery releases starting in 2003, and the 3-year-olds that returned to spawn in 2004 were in high abundance owing to this strong hatchery cohort (A.R. Murdoch, unpublished data). Neither weight nor run timing differed consistently between hatchery and wild fish, and neither of these traits explained the reduced fitness of hatchery fish.

Other than differences in age structure, carcass recovery location was the only measured trait that differed notably between hatchery- and wild-origin fish. Carcass recovery location also had a significant effect on fitness, such that fish that were recovered higher in the watershed had higher average fitness than those that were recovered lower in the watersheds. When spawning location was included as a predictor, the model coefficients associated with hatchery origin became less negative for both females and males and became nonsignificant for females in one of the two models. Overall, these results indicate that spawning location explains a portion but not all of the reduced fitness of hatchery fish in this study.

To our knowledge, this is the first direct estimate of the effect of general spawning location on fitness in a natural stream, although the result is consistent with previous indi-

rect observations, and there is a large literature on spawning site selection by salmon (reviewed by Quinn 2005). For example, Hoffnagle et al. (2008) found that hatchery Chinook salmon females were distributed lower in an Oregon watershed than wild females, and they hypothesized that this could have a deleterious effect on the fitness of the population. Schroder et al. (2008) found that small differences in spawning location within an artificial stream contributed to reduced fitness of hatchery spring Chinook salmon in the Yakima River.

The cause of the difference in spawning distribution in our study is probably due to the rearing methods and release location of the hatchery fish. Hatchery juveniles are initially reared in an offsite facility until 6–7 months of age, when they are transferred to a rearing pond near the mouth of the Chiwawa River. There, the fish are reared on a mixture of Chiwawa River and Wenatchee River water until they are released as smolts. Salmon are known to imprint on chemical cues in the water (Quinn and Fresh 1984; Quinn 1993). It is therefore not surprising that returning hatchery fish tend to spawn in the lower reaches of the Chiwawa River and areas nearby in Nason Creek and the Wenatchee River, since this is the area to which they imprinted as juveniles. Assuming that sufficient habitat capacity was available, releasing fish higher in the watershed might therefore result in improved fitness of the returning hatchery adults.

Our results do not directly address the mechanism by which spawning in the lower reaches of the Chiwawa River and Nason Creek leads to lower fitness, but there are two plausible factors. First, the density of spawners is higher in the lower reaches, owing to the large number of hatchery fish produced by the supplementation program. Second, the spawning and rearing habitat in the lower reaches are more impacted by roads and development (Upper Columbia Salmon Recovery Board 2007). Other aspects of the effect of spawning location remain to be explored. For example, relatively few wild fish but considerable hatchery fish spawned in the Wenatchee River proper, and this choice of spawning location may also contribute to the overall reduction in hatchery fish fitness.

One limitation of our study is that it does not directly estimate the genetic versus environmental components of differences between the hatchery and wild fish. In particular, the hatchery and wild spawners experienced different juvenile rearing environments, which could lead to environmentally induced differences in behavior or physiology that in turn lead to differences in fitness. The difference in spawning location between the two types of fish, for example, is probably due to differences in their early rearing environment. However, even after accounting for spawning location, hatchery fish remained less fit than wild fish (especially males), and some of those differences could be due to genetic effects or environmentally induced effects on traits we did not measure. For example, there have been studies that have found differences between hatchery fish in a variety of behavioral (e.g., Riddell and Swain 1991; Fleming and Gross 1992), morphological (e.g., Fleming and Gross 1989), and physiological traits (e.g., Hill et al. 2006).

We found larger effects of hatchery origin on fitness compared with some other recent studies of Chinook salmon in seminatural environments. In particular, Schroder et al. (2008) found that egg to fry survival for first generation hatchery females was 0.94 times that of wild females when measured in an artificial stream channel. There are several potential reasons for the greater differences in fitness in our study. First, the Wenatchee River supplementation program was started in 1989, and returning hatchery fish have comprised 36%–70% of the broodstock since 1994. The Yakima River program started in 1997 and uses only wild fish for broodstock, so the hatchery fish in the Wenatchee River have experienced more generations of domestication selection than the Yakima River fish. Second, we measured fitness over a longer period of the life cycle, allowing for more opportunities for differences in fitness between wild and hatchery fish to manifest. Third, a portion of the fitness differences we observed was due to large-scale differences in spawning location, which would not be able to be manifest in a spawning channel. Finally, the Wenatchee study was in a natural setting, compared with an artificial stream channel in the Yakima River. It is possible that the natural environment is less benign to hatchery-produced fish than the stream channel, leading to greater differences in fitness.

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The response of zooplankton in a whole-lake experiment on the effects of a cage aquaculture operation for rainbow trout (*Oncorhynchus mykiss*)

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Abstract: There is hope that increased development of aquaculture will help meet future global needs for protein. The growth of the freshwater aquaculture industry in Canada, however, has been hampered by insufficient information on environmental impacts. We examined the effects of an experimental cage aquaculture operation for rainbow trout (*Oncorhynchus mykiss*) on planktonic Crustacea and rotifers using 6 years of precage and 7 years of postcage data. Following the initiation of aquaculture, total crustacean and rotifer biomass, egg production, and depth distributions did not change significantly when compared with data from three nearby unimpacted lakes. We found statistically detectable increases in densities of *Bosmina* cf. *longirostris* and *Diacyclops thomasi* and an overall change in crustacean zooplankton community structure using correspondence analysis. The response of zooplankton was less than we expected because comparatively large changes were observed in phytoplankton biomass, hypolimnetic O_2, and densities of invertebrate (*Mysis diluviana*) and fish predators. Our study emphasizes the need to monitor multiple variables when assessing the ecosystem impacts of potential stressors such as aquaculture.

Résumé : On espère qu'une augmentation du développement de l'aquaculture aidera à combler les besoins globaux futurs de protéines. Cependant, la croissance de l'industrie de l'aquaculture en eau douce au Canada est entravée par le manque d'information sur les impacts environnementaux. Nous examinons les effets d'une opération expérimentale d'aquaculture de truites arc-en-ciel (*Oncorhynchus mykiss*) en cages sur les crustacés et les rotifères planctoniques à l'étude de 6 ans de données avant l'introduction des cages et de 7 ans après leur installation. Après le début de l'aquaculture, la biomasse totale des crustacés et des rotifères, leur production d'œufs et leur répartition en profondeur n'ont pas changé significativement par comparaison aux données provenant de trois lacs adjacents non affectés. Il y a eu des augmentations statistiquement décelables de densité de *Bosmina* cf. *longirostris* et de *Diacyclops thomasi* et une modification globale de la structure de la communauté de zooplancton d'après une analyse des correspondances. La réponse du zooplancton a été moins importante que prévu puisque des modifications comparativement grandes se sont produites dans la biomasse du phytoplancton, l'O_2 hypolimnétique, ainsi que dans les densités des invertébrés (*Mysis diluviana*) et des poissons prédateurs. Notre étude souligne la nécessité de surveiller plusieurs variables pour évaluer les impacts sur l'écosystème de facteurs potentiels de stress, tels que l'aquaculture.

[Traduit par la Rédaction]

Introduction

Although global demand for protein from fish is increasing, commercial catches from many wild fish stocks are stable or declining (Food and Agriculture Organization of the United Nations 2009). Hope exists that the gap between demand and wild fish supply can be filled by increasing production of cultured fish and by 2009, aquaculture production will likely exceed capture fisheries (Food and Agriculture Organization of the United Nations 2009). Although freshwater aquaculture is common in many countries (Food and Agriculture Organization of the United Nations 2009), the Canadian industry is small and primarily occurs in the North Channel of Lake Huron. Expansion of the freshwater cage aquaculture industry in Canada has been hampered by concerns about effects on the environment, and a lack of data on the impacts of freshwater cage aquaculture has limited effective government regulation.

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Previous studies show that cage aquaculture operations can cause increased nutrient loading and elevated growth of phytoplankton and bacteria (reviewed by Yan 2005; Podemski and Blanchfield 2006). Excessive growth of phytoplankton may lead to reduced water clarity, noxious algal blooms, and in extreme cases, hypolimnetic oxygen (O_2) depletion, especially when cages are in locations with limited water circulation. Where cages occur, wild fish frequently show increased rates of growth because of greater lake productivity and direct consumption of farm waste.

In Canada and elsewhere, regulators are committing to an ecosystem management approach that requires the identification of potentially important pathways of effect and of ecosystem indicators of impacts (e.g., Fisheries and Oceans Canada 2007). Using this approach, it is important to understand the effects of cage aquaculture on zooplankton because they serve as food for native fish and influence phytoplankton dynamics.

Our objective was to examine the effects of an experimental aquaculture operation on planktonic crustacean and rotifer biomass, species composition, egg production, and depth distribution. No previous studies have examined changes in freshwater zooplankton before and after the installation of a cage aquaculture operation. We summarize 13 years of data collected over a 22-year period in our study lake and 21–22 years of data from each of three unimpacted reference lakes located nearby. Following the initiation of the cage farm operation for rainbow trout (*Oncorhynchus mykiss*), concentrations of many nutrients, phytoplankton, and densities of fish increased (Bristow et al. 2008; Mills et al. 2008; Findlay et al. 2009). Numbers of benthic invertebrates, as well as an important invertebrate zooplankton predator, *Mysis diluviana* (formerly *Mysis relicta* (Audzijonyte and Vainola 2006), and hereafter referred to as *Mysis*), declined along with hypolimnetic O_2 concentrations (Paterson et al. 2010). Similar changes were not observed in the reference lakes. As a result of the changes in limnological conditions, we expected some or all of the following responses of zooplankton: (*i*) increased zooplankton biomass or turnover as a response to increased phytoplankton food availability; (*ii*) decreases in numbers of larger zooplankton such as *Daphnia* as a result of increased fish predation; (*iii*) increases in zooplankton taxa susceptible to *Mysis* predation; and (*iv*) changes in zooplankton depth distributions as a response to decreases of hypolimnetic O_2 concentrations and densities of *Mysis*, which preferentially inhabits the hypolimnion.

Materials and methods

Study sites

We examined long-term data from the site of the experimental cage aquaculture operation, Lake 375 (L375), and from three unimpacted reference lakes, Lakes 239, 373, and 442 (L239, L373, and L442) at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (Table 1). All are oligotrophic, dimictic lakes with average summer thermocline depths of 4–6 m. The outflow from L373 flows directly into L375 and Lakes 442 and 239 are located within 10 km. All four lakes contain populations of *Mysis* and several fish species, including lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersonii*), slimy sculpin (*Cottus cognatus*), and several cyprinid taxa (Mills et al. 2002). L239 also contains yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*). Boreal forest dominated by jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), and black spruce (*Picea mariana*) surrounds all of these undeveloped lakes.

The experimental cage farm was installed in L375 in 2003 and consisted of a single 10 m × 10 m × 10 m cage anchored in water 15 m deep (Bristow et al. 2008). Each year for 5 years, we added approximately 10 000 female fingerlings of rainbow trout to the cage in May and grew them until November. We fed fish twice daily with Martin Mills Profishent pellets, and each fall a total of approximately 10 tonnes of trout were harvested from the cage. In 2008, the cage was left empty.

Lake sampling

We sampled every 2 weeks during the open-water season for water chemistry, phytoplankton, zooplankton, and temperature. We sampled L375 from 1987 to 1992 and from 2001 to 2008 (7 years precage and 6 years postcage), whereas we sampled the three reference lakes every year from 1969 (L239) or 1988 (L373, L442). Although there was an 8-year gap in the record for L375, we believe the data collected between 1987 and 1992 provides a useful extension of the before aquaculture data set, increasing the power of our analyses. Below, we consider only data collected from the reference lakes since 1987 to facilitate comparisons with L375. We collected samples for water chemistry at the deep station of each lake at 5 m intervals using a peristaltic pump, and samples were analysed by the ELA Chemistry Laboratory using the methods of Stainton et al. (1977).

Although zooplankton sampling methods were consistent within each lake, different approaches were used in L239 and L375 compared with L442 and L373 (Table 1). Lakes 239 and 375 were sampled at the deep station during the day in the ice-free season using two vertical hauls of a double-barrelled closing 53 μm plankton net, where each barrel was 13 cm in diameter (Chang and Malley 1987). Beginning in 2001, we also sampled zooplankton in L375 at a second station (depth = 15 m) located near the cage (see Findlay et al. 2009 for a map of sample site locations). This station was sampled to examine whether current regulations that require sampling near cages in Lake Huron provide an adequate measure of aquaculture impacts on zooplankton.

Lakes 442 and 373 were sampled during the day for zooplankton at seven pelagic stations in each lake using a tube sampler constructed from a flexible PVC hose calibrated for volume with a weighted 7.6 cm diameter Plexiglas pipe and retrieval cord attached to the end (Salki 1993). At each station, we lowered the sampler to approximately 0.25 m above the lake bottom, brought it to the surface, and poured the contents through a net. Between 1988 and 1997, samples were filtered through a 72 μm net; thereafter, a 53 μm net was used to provide consistency among all study lakes at ELA. Comparisons of counts with the 72 and 53 μm nets revealed that Crustacea were sampled with equal efficiency by both nets, but many rotifers retained on the 53 μm net passed through the 72 μm mesh. As a result, we only examined rotifer data collected after 2001. Lake 442 was not used

Table 1. Morphometry, locations, and zooplankton methods used in each of the study lakes.

Lake	Area (ha)	Maximum depth (m)	Volume ($\times 10^5$ m^3)	Latitude, longitude	Zooplankton sampling method
375	23	26	21.7	49°44′43.61″N, 93°47′15.56″W	Net hauls at center buoy (second station added in 2001 near the aquaculture cage)
239	56	30	59.1	49°39′45.55″N, 93°43′21.68″W	Net hauls at center buoy
373	27	20	29.4	49°44′41.46″N, 93°47′55.35″W	Tube sampler at multiple stations
442	16	18	13.0	49°46′32.18″N, 93°49′3.27″W	Tube sampler at multiple stations

in the analyses of rotifer data because they were not sampled in this lake until 2003.

We preserved all samples in 4% sugar–formalin and counted zooplankton using the methods of Malley and Chang (1994), except that c4 and c5 copepods were identified to species after 1994. We calculated the biomass of each species (μg dry mass·L^{-1}) by multiplying population density (individuals·L^{-1}) by mean individual biomass (μg dry mass·individual^{-1}). Individual species biomasses were estimated from length measurements and length–weight published regression equations for cladocerans and copepods (Lawrence et al. 1987; Malley et al. 1989) and published individual masses for rotifers (Schindler and Noven 1971).

We examined the vertical distribution of crustacean zooplankton during the day and night in L375 in late July or early August in 2002 (precage), 2003, 2007, and 2008 (postcage). Samples were collected at 2 m intervals at the deep station using a 28 L Schindler–Patalas trap with a 53 μm net. Night samples were collected at least 1 h after sunset. Samples were preserved and counted as described above.

To examine possible changes in zooplankton population turnover, we determined egg ratios of common zooplankton in reference lakes L239 and L373 and for our study lake, L375, for the years 2001–2008. We were unable to convert egg ratios into birth rates using egg development times based on temperature because our samplers integrated zooplankton densities throughout the entire water column. As a result, the specific water depths where different taxa resided and their associated temperatures were unknown. Nonetheless, we believe that egg ratios provide a crude comparative proxy for among-year changes in per-capita birth rates because seasonal and vertical temperature regimes and diurnal migration patterns did not change substantially before and after the installation of the cage. Egg ratios for copepod taxa utilized only adult females, whereas estimates for female cladocerans did not account for changes in size or age distributions. Loose eggs for each crustacean group (Cyclopoids, Calanoids, Cladocera) were apportioned among taxa based on the relative abundance of females within each group. Only dates where at least five females and their eggs were counted were included in the analysis.

Statistical analysis

We used a before–after, control–impact (BACI) design to compare the differences between monthly zooplankton densities in L375 and the mean density for the three reference lakes (Stewart-Oaten et al. 1986; Stewart-Oaten et al. 1992). For crustacean data, we utilized the 7 common years of precage data (1988–1992, 2001–2002) and the 6 years of postcage data (2003–2008) in our analysis. For rotifers and crustacean egg ratios, we utilized monthly data from 2 precage years (2001–2002) and 6 postcage years (2003–2008). All data were $\log_{10}$-transformed before using a t test to compare differences before and after establishment of the cage farm operation. We adjusted probability values for multiple comparisons using a standard Bonferroni correction on a table-wise basis. For example, a separate Bonferroni correction was used in the comparisons for different taxa of Crustacea and rotifers.

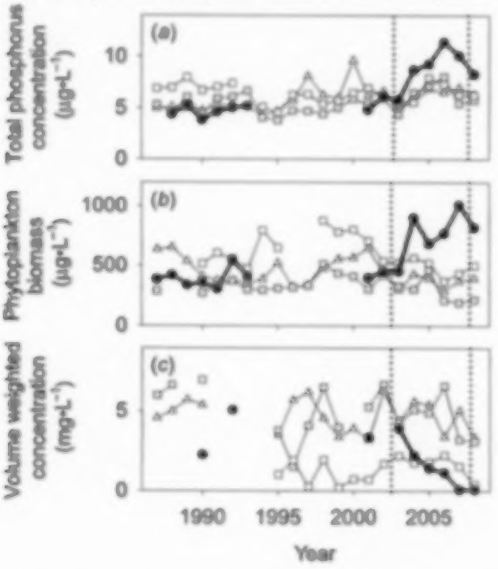
Changes in annual average species composition for crustacea and rotifers were examined using correspondence analysis (CA; Hill 1974). Density data (number·L^{-1}) were $\log_{10}$-transformed, and only taxa that represented >5% of total abundance in each group were included. The resulting orthogonal axis scores for each lake-year were then tested for differences before and after the installation of the cage using a BACI analysis as described above. All statistical analyses were completed using SYSTAT version 12 (Systat Software Inc., Chicago, Ill.).

Results

Limnological conditions

Substantial limnological changes occurred in L375 following establishment of the cage aquaculture operation in 2003 (Fig. 1). Average epilimnetic total phosphorus (TP) concentrations increased from 4.9 to >10 μg·L^{-1} and phytoplankton biomass from 398 μg wet mass·L^{-1} to as high as 1000 μg wet mass·L^{-1} (Bristow et al. 2008; Findlay et al. 2009). In particular, large increases in epilimnetic TP and phytoplankton densities (>15 μg·L^{-1} and >5000 μg wet mass·L^{-1}, respectively) were observed during lake mixing in May and early June (Findlay et al. 2009). Using L373 as a reference, analyses by Findlay et al. (2009) indicated statistically significant changes in TP and phytoplankton biomass following installation of the cage farm. After 2006, average hypolimnetic O_2 concentrations in September decreased to below 1 mg·L^{-1} in L375. In association with this decline, *Mysis* densities decreased from an average of 88·m^{-2} between 2002 and 2005 to 6·m^{-2} in 2008 (Paterson et al. 2010). There were also increases in fish densities and growth rates (Mills et al. 2008). Although no rainbow trout were farmed in 2008, most limnological conditions were similar to 2007 (Fig. 1). Average limnological conditions in the three reference lakes did not change dramatically over the 22-year period of study, with one exception. *Mysis* den-

Fig. 1. Limnological conditions during the open-water season including mean annual time-weighted epilimnetic concentrations of (*a*) total phosphorus, (*b*) total phytoplankton biomass (wet mass), and (*c*) mean volume-weighted hypolimnetic O_2 concentrations in September. Symbols are as follows: L375 — solid circles, L239 — gray triangles, L373 — gray squares, L442 — open squares. The dotted vertical lines depict the beginning and end of cage farming in L375 in 2003 and 2008, respectively. Missing data points for O_2 measures occur because not all depths were sampled from each lake in every year. Parts of these data sets were previously published in Findlay et al. (2009) and Paterson et al. (2010).



sities also declined in L373 in 2006–2008, but the decrease was not as dramatic as in L375 (from 83 to 38·m^{-2}).

Crustacea

Lake 375 and the reference lakes had similar crustacean zooplankton communities and densities. Along with unidentified early-stage copepods, seven taxa accounted for >90% of the crustacean biomass in all lake-years. These were the cladocerans *Bosmina* cf. *longirostris*, *Daphnia galeata mendotae*, *Diaphanosoma birgei*, and the copepods *Diaptomus minutus*, *Diaptomus sicilis* (excepting Lake 239), *Diacyclops thomasi*, and *Tropocyclops extensus*.

Crustacean zooplankton were sampled in all lakes in 12 of the 22 years of collections (Fig. 2). Following establishment of the aquaculture operation in L375, significant increases in densities were observed for *Bosmina* cf. *longirostris* (BACI *t* test; df = 66; $p < 0.005$ following Bonferroni correction for analyses of seven crustacean taxa) and *Diacyclops thomasi* (df = 66; $p < 0.005$). Densities of *Tropocyclops extensus* also increased in L375 in 2007 and 2008, but postcage changes were not statistically significant when considering the full period of operation (df = 66; $p < 0.3$). Although densities of *Diaptomus minutus* did not change dramatically in L375, the average difference from the reference lakes significantly increased during the period of cage operation (df = 66; $p < 0.005$). This was because of a long-term trend of increasing abundance in the reference systems that was not observed in L375. A significant change in total crustacean biomass was not detected (df = 66; $p < 0.8$). For all crustacean zooplankton taxa, no significant differences in densities between the north and south basins of L375 were observed before or after establishment of the cage farm (df = 45; $p < 0.9$)

Axes 1 and 2 of the CA ordination for Crustacea explained 34% and 16%, respectively, of the variance in the species by lake-year data matrix (Fig. 3*a*). Lake-years with lower scores on Axis 1 included all years from L373 and L442 and from L375 prior to establishment of the cage farm. These lake-years had comparatively higher densities of *Diaptomus sicilis* and lower densities of *Bosmina* cf. *longirostris*, *Tropocyclops extensus*, and *Diacyclops thomasi* (Fig. 3*b*). Samples from L239 and L375 after establishment of the cage operation had higher scores on Axis 1 of the CA ordination (Fig. 3*a*). Lake-years with lower scores on Axis 2 had higher densities of *Tropocyclops extensus*. Differences between the scores on Axis 1 for Lake 375 and the reference lakes changed significantly following establishment of the cage farm (Fig. 3*c*; BACI *t* test, df = 10; $p < 0.02$). There is a suggestion that species composition may have been returning to precage conditions in 2008, the first year when rainbow trout were not added to the cage. Differences in Axis 2 scores before and after establishment of the cage farm were not statistically significant (df = 10; $p < 0.9$).

Seasonal patterns of abundance did not change dramatically for most taxa following establishment of the cage farm in 2003 (Supplementary Fig. S1[2]). Exceptions included increased abundances of *Bosmina* cf. *longirostris*; c1–c3 cyclopoid copepods in the spring of 2006, 2007, and 2008; and *Tropocyclops extensus* in the fall of 2006 and 2007. Establishment of the cage farm in L375 also did not strongly affect egg ratios of common crustacean zooplankton taxa (Supplementary Fig. S2[2]), and no statistically significant changes were detected for the postcage versus precage periods for any taxon. In 2007 and 2008, egg ratios for *Bosmina* cf. *longirostris* increased considerably in May, as compared with previous years.

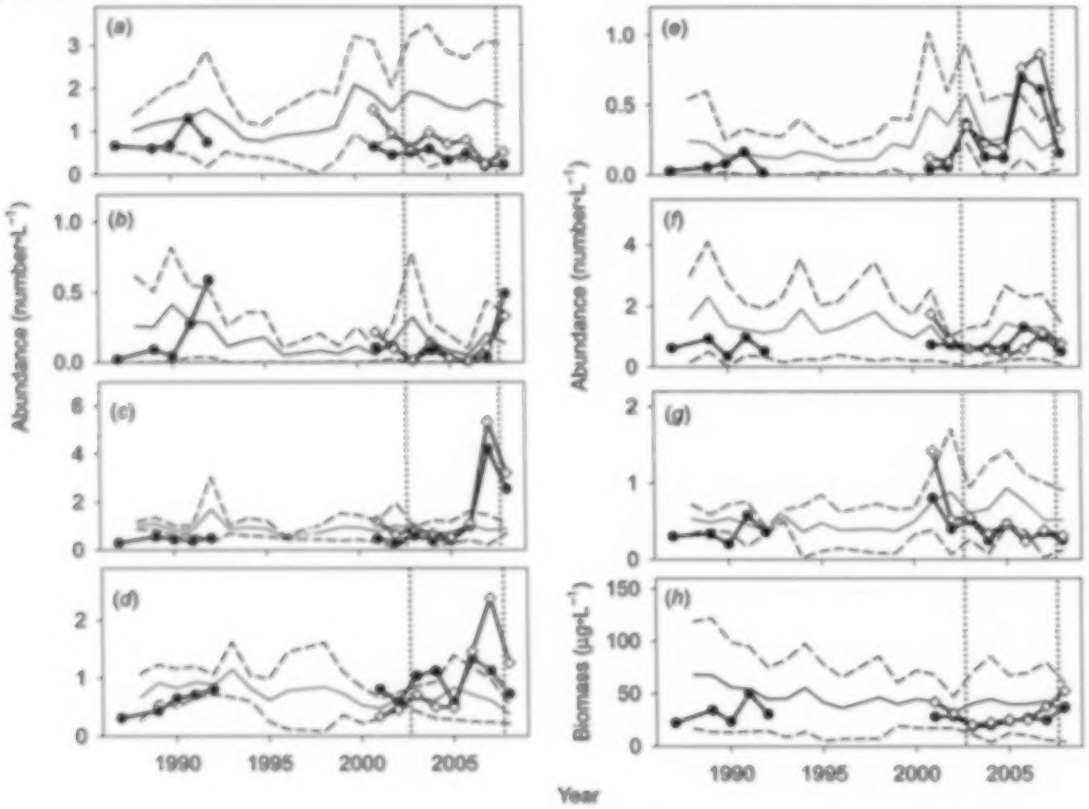
Rotifers

Ten taxa comprised >95% of rotifer biomass in all lake-years. These were *Collotheca* sp., *Conochilus unicornis*, *Gastropus stylifer*, *Kellicottia bostoniensis*, *Kellicottia longispina*, *Keratella cochlearis*, *Keratella testudo*, *Polyarthra remata*, *Polyarthra vulgaris*, and *Synchaeta* sp. After a Bonferroni correction for comparisons of 10 taxa, we found that densities of no rotifer taxa changed significantly following establishment of the cage operation in 2003 (Supplementary Fig. S3[2]). There was also no significant change in total rotifer biomass (df = 48; $p < 0.2$). Installation of the cage farm did not significantly affect average differences between densities in the north and south basins (df = 48; $p < 0.6$).

Axes 1 and 2 of the rotifer CA explained 36% and 22%, respectively, of the variance in the lake-year by density matrix (Fig. 3*d*). Axis 1 separated lake years with higher den-

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 2. Mean annual time-weighted abundances of common crustacean zooplankton taxa and total zooplankton biomass in L375 north basin (solid circles) and L375 south basin (open diamonds): (*a*) *Diaptomus minutus* adults, (*b*) *Diaptomus sicilis* adults, (*c*) *Bosmina* cf. *longirostris*, (*d*) *Diacyclops thomasi* adults, (*e*) *Tropocyclops extensus* adults, (*f*) *Daphnia galeata mendotae*, (*g*) *Diaphansoma birgei*, (*h*) total zooplankton biomass. Annual averages for species' abundances and total zooplankton biomass in the three reference lakes (L239, L373, L442) are indicated with the solid line. Dashed lines indicate ±1 standard deviation among reference lakes. Reference lake means for *Diaptomus sicilis* do not include L239, where this species does not occur. The dotted vertical lines depict the beginning and end of cage farming in L375 in 2003 and 2008, respectively.



sities of *Kellicottia bostoniensis* from those with higher densities of *Gastropus stylifer* and *Polyarthra remata*, while Axis 2 separated lake years with higher densities of *Polyarthra remata* and *Kellicottia bostoniensis* from those with greater representation by *Keratella testudo* and *Gastropus stylifer* (Figs. 3*d*, 3*e*). Differences in scores for L375 and the reference lakes on Axes 1 and 2 were not statistically different following establishment of the cage farm (df = 6; $p < 0.5$ for Axis 1, $p < 0.6$ for Axis 2; Fig. 3*f*).

Vertical migration of crustacean zooplankton in L375

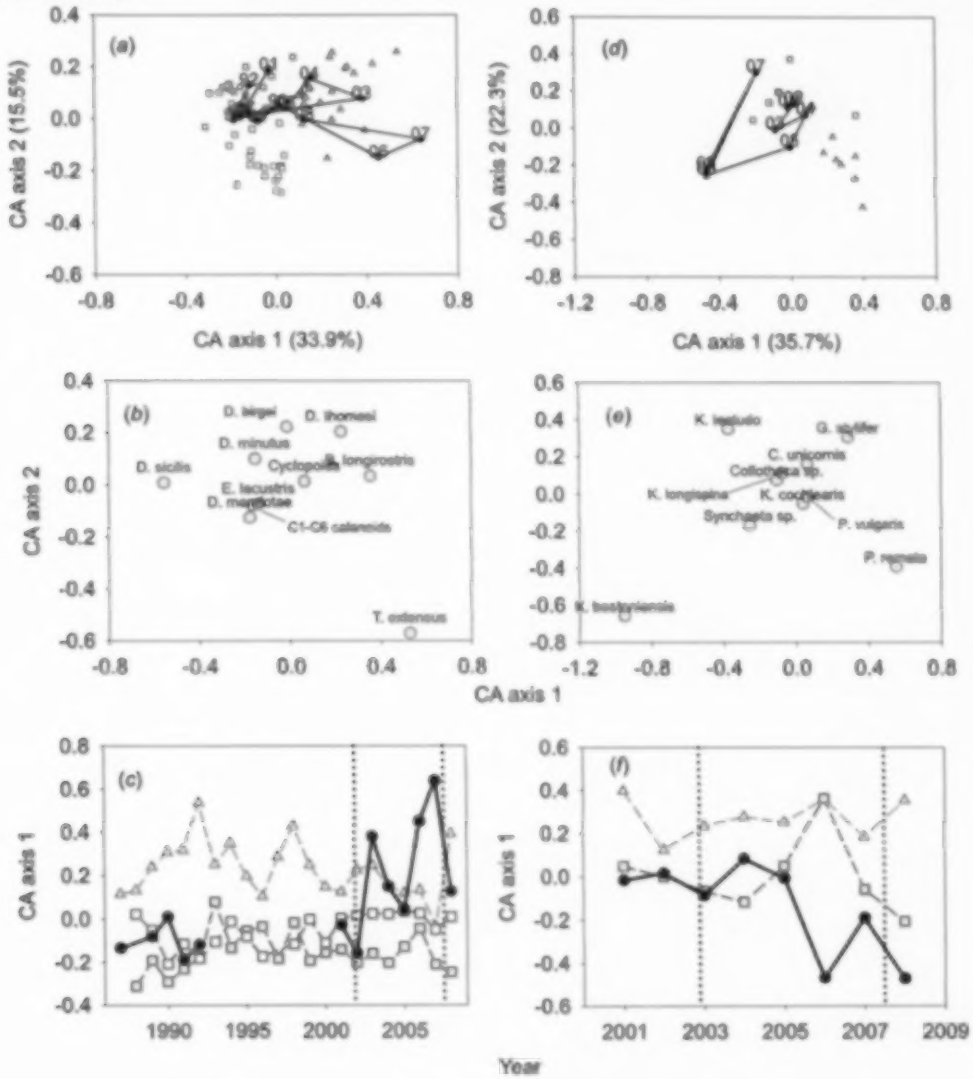
The vertical distribution of crustacean zooplankton in late July and early August did not change substantially after cage installation in 2003 (Figs. 4*a*–4*d*). In all years that were sampled, the highest densities occurred immediately below the thermocline and with an associated deep chlorophyll peak (Figs. 4*e*–4*h*). Densities of all taxa were low at depths greater than 15 m both before and after establishment of the cage farm. There was some evidence of upward migration of Crustacea at night, but diurnal changes in mean depths of total biomass and of densities of common taxa were generally less than 2 m.

Discussion

Total crustacean and rotifer biomass, crustacean egg production, and zooplankton depth distributions did not detectably change following the establishment of the aquaculture operation in L375. The greatest changes we observed were in species composition where we found statistically detectable increases in densities of *Bosmina* cf. *longirostris* and *Diacyclops thomasi* and an overall change in crustacean zooplankton community structure as measured using correspondence analysis (CA). Overall, however, the responses of the zooplankton community (including both microcrustacea and rotifers) to aquaculture were less than we expected based on increases in phytoplankton and native fish after 2004 and decreases in hypolimnetic O_2 concentrations and *Mysis* after 2006.

Many of the changes in limnological conditions and food web structure that we saw in L375 are similar to changes that may occur as a result of other stressors. For example, *Mysis* densities may decline as a result of acidification (Nero and Schindler 1983), eutrophication (Scharf and Koschel 2004), or climate change (Schindler et al. 1996).

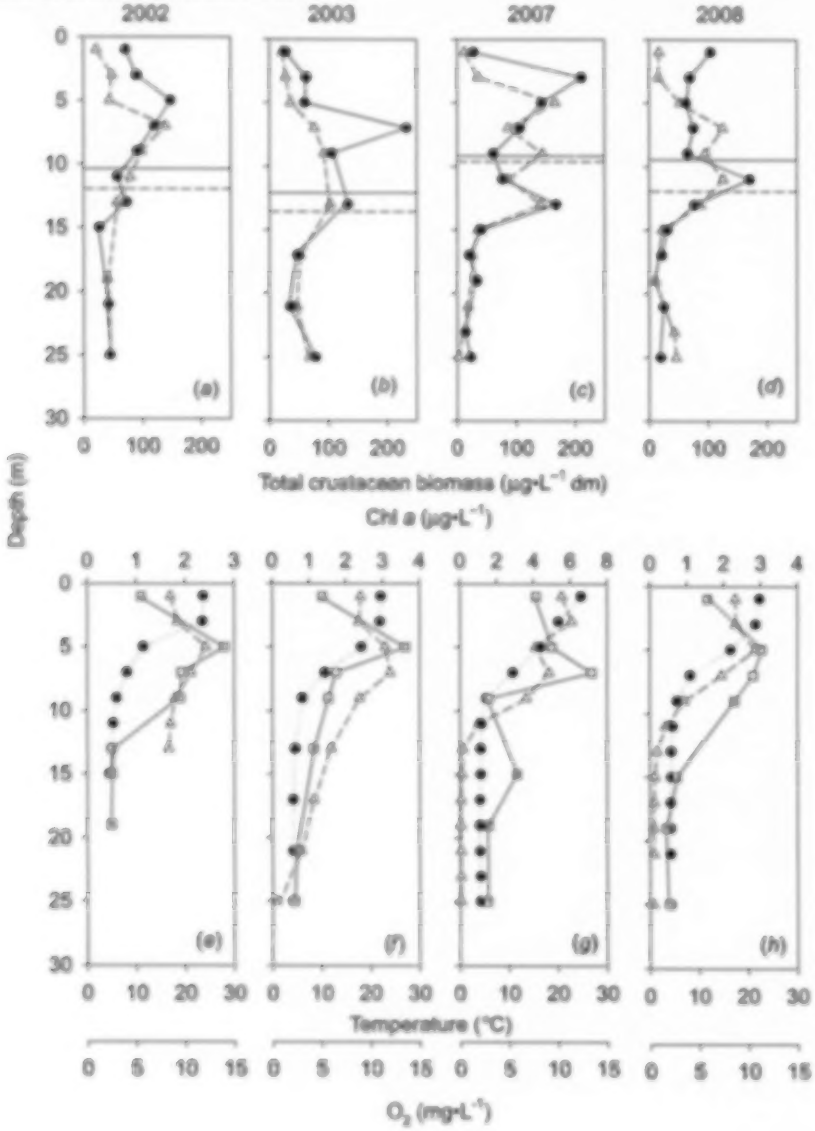
Fig. 3. (*a*) Correspondence analysis (CA) scores for lake-years using data for common crustacean taxa. Labels denote different lake-years in L375. (*b*) CA scores for common crustacean taxa. (*c*) Changes in crustacean CA scores for Axis 1 in L375 and the three reference lakes. (*d*) CA scores for lake-years using data for common rotifer taxa. (*e*) CA scores for common rotifer taxa. (*f*) Changes in rotifer CA scores for Axis 1 in L375 and the three reference lakes. Symbols for lake years are as follows: L375 — solid circles, L239 — gray triangles, L373 — gray squares, L442 — open squares. In panels (*c*) and (*f*), the dotted vertical lines depict the beginning and end of cage farming in L375 in 2003 and 2008, respectively.



Changes in phytoplankton biomass, hypolimnetic O_2 concentrations, and fish growth are typical of eutrophied lakes (Schindler and Vallentyne 2008). Consequently, the results from L375 also provide insight into the potential impacts of other stressors.

With the development of greater phytoplankton densities in L375 after 2004, we expected to see concomitant increases in numbers and biomass of zooplankton. Among-lake surveys of zooplankton and phytoplankton biomass typically find strong relationships between the two groups, and log–log regressions often have slopes near 1 (e.g., McCauley and Kalff 1981; Hanson and Peters 1984). As a result, we expected that zooplankton biomass would increase approximately proportionally with increases in phytoplankton in L375. Despite a greater than twofold increase in phytoplankton biomass from a precage average of 398 to 1008 µg wet mass·L^{-1} in 2007, there were no large changes in total zooplankton biomass. Egg ratios of common crustacean taxa also did not increase, further suggesting that food availability and population turnover did not change substantially. Although among-lake surveys typically show strong relationships between zooplankton and phytoplankton densities, within-lake studies are often less equivocal. Following lake eutrophication, some studies have observed increases in zoo-

Fig. 4. (*a*–*d*) Depth distribution zooplankton biomass during the day (open triangles) and night (closed circles) in late July or early August in (*a*) 2002, (*b*) 2003, (*c*) 2007, and (*d*) 2008. Weighted mean depths for zooplankton biomass are depicted with solid lines and dashed lines for night and day, respectively. (*e*–*h*) Depth distributions of dissolved O_2 (open triangles), temperature (solid circles), and chlorophyll *a* (gray squares) in (*e*) 2002, (*f*) 2003, (*g*) 2007, and (*h*) 2008.



plankton biomass, densities, or turnover (e.g., Patalas and Salki 1973; Blomqvist et al. 1993), but others have reported little or no change (e.g., Neill 1988; Carpenter et al. 1995), consistent with our results.

In L375, nutrients introduced by the cage farm primarily accumulated in the hypolimnion during the stratified period and only became available to phytoplankton during periods of lake mixing (Findlay et al. 2009). This resulted in blooms of algae that persisted for only a few days or weeks in the spring and autumn. During the stratified season, increases in phytoplankton were not as great and primarily in inedible size fractions (linear dimension >20 µm). In the spring and autumn, zooplankton densities were typically low, possibly because of cool temperatures or predation, and herbivore populations were apparently unable to fully utilize the briefly available resources. Possible exceptions to this were *Bosmina* cf. *longirostris* and *Tropocyclops extensus*, which had increased densities and egg ratios in the spring and autumn, respectively, in 2006–2008.

After 2006, densities of *Mysis*, the most abundant invertebrate predator of zooplankton in L375, decreased substantially, along with declines in hypolimnetic O_2

concentrations. Introductions of *Mysis* to many lakes have resulted in dramatic impacts to native zooplankton communities (reviewed by Lasenby et al. 1986), and we expected that declines of this predator might also have important effects in L375. Because *Mysis* does not inhabit waters with temperatures above 10–15 °C, it typically has the greatest impact on zooplankton that inhabit the hypolimnion or predominate during lake mixing in spring and autumn (e.g., Martinez and Bergersen 1988; Spencer et al. 1999). Decreases in densities of *Mysis* in L375 did not result in higher densities of zooplankton during lake mixing, with the possible exceptions of *Bosmina* cf. *longirostris* and *Tropocyclops extensus*. Several studies have indicated that *Bosmina* is especially susceptible to predation by *Mysis* (Koksvik et al. 1988; Langeland et al. 1988; Spencer et al. 1999). Examination of vertical distributions of crustacean zooplankton in L375 indicated that zooplankton densities were generally low in the hypolimnion both before (2002, 2003) and after (2007, 2008) the decline of *Mysis* and hypolimnetic O_2 concentrations. Prior to 2006, *Mysis* predation may have excluded many zooplankton from the hypolimnion, and in later years they may have been unable to move to deeper depths because of the negative effects of low O_2 concentrations. In all years except 2008, *Diacyclops thomasi* was the dominant taxon in samples collected below the thermocline in L375. As a result, increased densities of *Diacyclops thomasi* after installation of the cage farm may have been a response to decreased *Mysis* predation.

In 2004, densities of fathead minnows (*Pimephales promelas*) and other cyprinids approximately doubled (Mills et al. 2008), and in subsequent years, densities and growth rates of cyprinids, lake trout, and sculpins increased even more (K. Mills and P. Blanchfield, Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada, unpublished data). Additionally, approximately 10 000 rainbow trout were added each year to the aquaculture cage. Although these fish primarily relied on artificial fish feed, even limited zooplankton consumption could potentially be important because of the extremely high density of stocked fish relative to the native fish community. The absence of significant differences in zooplankton densities estimated near the cage and farther away in the south basin argues against this latter possibility, however.

Because changes in fish densities have been frequently shown to have dramatic effects on zooplankton, the absence of a strong response in L375 is surprising. In part, this may be because the majority of fish in the lake did not rely heavily on zooplankton as a food resource. Examination of stable isotopes of C and N suggested that cyprinids primarily utilized littoral invertebrates, and zooplankton contributed less than 30% of their overall diet (Kullman et al. 2009). Possibly, increases in predation by fish were partially offset by decreases in *Mysis* predation. Although exposure to fish kairomones often results in changes in zooplankton migratory behaviour in the laboratory (e.g., Dodson 1988), there was no evidence that the annual introduction of rainbow trout affected zooplankton vertical distributions in L375.

An important limitation of our study is that it was undertaken in only a single study lake. Although small lakes have been used for cage aquaculture in Europe and elsewhere (e.g., Kelly 1995), L375 is much smaller and has a longer water residence time than sites that would be typically approved for a cage aquaculture operation in Canada. Consequently, the impacts of the farm operation in L375 were much greater than would be expected for commercial operations in larger lakes (for further discussion, see Bristow et al. 2008; Findlay et al. 2009). Despite the comparatively large changes in water chemistry and food web structure observed in L375, zooplankton community structure did not change dramatically. This suggests that our results are conservative with respect to the potential impacts of commercial freshwater aquaculture operations on zooplankton in larger lakes. It is essential, however, that further studies be undertaken in other lakes with different limnological conditions to fully understand the range of potential impacts of freshwater aquaculture on zooplankton. To our knowledge, there are no comparable published data with which to compare the L375 results. In our study, there was little evidence that changes in zooplankton affected phytoplankton development or fish growth and survivorship in L375.

While it is clear that zooplankton strongly respond to stressors on many occasions, our results indicate that this is not always the case. With the possible exception of increases in *Diacyclops thomasi* and *Bosmina* cf. *longirostris* as a response to declining *Mysis* predation, none of our expectations concerning likely changes in zooplankton community structure were realized. Zooplankton biomass and turnover did not increase with greater phytoplankton densities, numbers of larger zooplankton taxa (e.g., *Daphnia*) did not decrease with increased fish densities, and zooplankton depth distributions did not change in response to changes in hypolimnetic O_2 concentrations, *Mysis*, or fish densities. Overall, changes in zooplankton community structure in L375 were small, as compared with changes in other limnological conditions and food web structure. To us, this was surprising given the central role that zooplankton play in pelagic planktonic community structure. Our results emphasize that a wide variety of different variables must be monitored to fully quantify the ecosystem impacts of potential stressors such as aquaculture. The selection of the best variables to monitor will depend on the expected impacts of a given stressor, based on studies like the one in L375 and elsewhere, and on important pathways linking stressors with variables of concern, such as fish or water quality.

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Analysis of variables associated with the *Ichthyophonus hoferi* epizootics in Norwegian spring spawning herring, 1992–2008

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Abstract: We present a long-term study of epizootics in a migratory fish species that shows major advancements in our understanding of marine disease ecology. On the individual macroscopic level, *Ichthyophonus hoferi* infections in herring significantly reduced body condition ($p < 0.01$) and reproductive capacity (gonad weight, $p < 0.001$). Infection prevalence increased with age ($p < 0.001$, $R^2_{adj} = 0.71$). Prevalence in the catches strongly depended on target school size and was thus higher in trawl catches. We found strong seasonality in infection prevalence within years, with peaks in summer and winter. Summer peaks spatially formed an "infectious belt" off the west coast of Norway, consisting of infected fish unable to follow the migration routes to the feeding grounds. Prevalence varied also between years ($p \leq 0.001$). Since 1992, it had declined from stock prevalence levels of 10%, down to almost extinction, with another major peak in 1999. The annual peaks could best be associated with strong year classes (Pearson's $r = 0.56$), a good overall body condition ($r = 0.86$), and plastic wintering behaviour.

Résumé : Nous présentons une étude à longue haleine d'une épizootie chez une espèce de poisson migrateur qui représente des progrès importants dans notre compréhension de l'écologie des maladies marines. À l'échelle macroscopique individuelle, les infections à *Ichthyophonus hoferi* chez le hareng réduisent significativement la condition corporelle ($p < 0{,}01$) et la capacité reproductive (masse des gonades, $p < 0{,}001$). La prévalence de l'infection augmente en fonction de l'âge ($p < 0{,}001$, $R^2_{adj} = 0{,}71$). La prévalence dans les captures est fortement reliée à la taille du banc de poissons ciblé et est ainsi plus importante dans les récoltes au chalut. Il existe une forte saisonnalité dans la prévalence de l'infection au cours d'une même année, avec des maximums en été et en hiver. Les maximums d'été forment dans l'espace une « ceinture infectieuse » au large de la côte ouest de la Norvège, composée de poissons infectés incapables de suivre les routes de migration vers les aires d'alimentation. La prévalence varie aussi d'une année à l'autre ($p \leq 0{,}001$). Depuis 1992, le niveau de prévalence de l'infection a diminué de 10 % du stock à presque l'extinction pour atteindre un nouveau maximum en 1999. Les pics annuels peuvent être le mieux associés aux classes d'âge fortes (r de Pearson = 0,56), à une bonne condition corporelle globale ($r = 0{,}86$) et à un comportement flexible d'hivernage.

[Traduit par la Rédaction]

Introduction

Progress in marine ecology needs an understanding of the fundamental processes underlying marine disease dynamics (e.g., Swinton et al. 1998; Jolles et al. 2002; Kim and Harvell 2004). *Ichthyophonus hoferi* in Atlantic herring (*Clupea harengus*) is an example of a pathogen in an economically important, migrating fish stock that has been known since 1893 to cause recurrent epizootics with major losses, mainly in the western North Atlantic (Sindermann 1956; Rahimian and Thulin 1996; McVicar 1999). Recently, the disease has also been described in Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska (Marty et al. 1998), and in Puget Sound, Washington (Hershberger et al. 2002).

Ichthyophonus hoferi is a nonspecific, fungus-like protozoan, best described as a member of the class Mesomycetozoea, which causes systemic infections in 80–100 species of fishes, amphibians, and crustaceans (Rahimian and Thulin 1996; Mendoza et al. 2002). The infection is often lethal in herring, and clinical studies have shown that herring survive for 1–3 months after visible clinical signs have developed, with maximum survival being about 6 months (Sindermann 1958; McVicar 1981). In August 1991, the disease was detected in the North Sea autumn spawning stock between Denmark and Sweden and also in the Norwegian spring spawning herring (NSSH) feeding in the Norwegian Sea (Hjeltnes and Skagen 1992; Holst 1992). It was hypothesized that the epizootic had its origins in the European plaice (*Pleuronectes platessa*) mass mortality caused by *I. hoferi* off the Scottish coast (McVicar 1981; Rahimian and Thulin 1996). Since then, programs have been launched to monitor disease prevalence patterns. In autumn 2008, a

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new outbreak of *I. hoferi* that displayed a prevalence of up to 50% in purse seine catches was reported in Icelandic summer spawning herring (Oskarsson and Palsson 2009).

We synthesize and condense the unique long-term data set of *I. hoferi* monitoring from 1992 to 2008 in NSSH to generate new hypotheses regarding the epidemiology of the disease. We aim to identify general patterns and to discuss the possible mechanism generating those patterns. To this end, we first describe individual physiological differences in infected and noninfected fish. We then investigate possible disease drivers on the population level in a spatio-temporal context. A special challenge is presented by the use of spatial epidemiological techniques for migrating species and accounting for seasonality. Seasonality influences abiotic factors like sea water temperature that in turn affect disease prevalence pattern (Harvell et al. 1999). Seasonality in migration also changes feeding habits and stock density. For example, from terrestrial systems it is known that density plays an important role in disease dynamics (Anderson and May 1979; Grenfell and Dobson 1995). We close by providing practical guidelines on how to improve the sampling scheme in marine epizootics that arise after analysing this data set.

The Norwegian spring spawning herring stock

The NSSH is a highly migrating herring stock in the northeast Atlantic (Supplemental Appendix Fig. S1[3]). Its main distribution is in the Barents Sea as juveniles and in the Norwegian Sea as adults. The NSSH spawn demersal eggs in early spring along the Norwegian west coast from about 62°N to 69°N. The main larval drift is northwest, along the Norwegian coast. Some larvae end up in fjords along the coast, while the majority of the year classes end in up the Barents Sea, where they spend from 3 to 6 years, depending on growth conditions. At a size of around 20–23 cm, the young herring leave the Barents Sea and spend a year or two outside the area of the County of Troms before the onset of maturation at around 27 cm. The southwesterly spawning migration along the Norwegian coast is size-dependent, and the largest fish are found in the southernmost areas. After spawning, the herring spread out into the feeding areas between the Norwegian coast, the Faeroes, Iceland, Jan Mayen Island, and Bear Island. The main feeding and condition gain takes place during a hectic period of about 2 months from late May to mid-July, when the herring experience large plankton concentrations near the polar front areas. After the summer feeding period, the stock contracts and concentrates in the wintering areas. In 1972–2002, the main wintering areas were in fjords inside the Lofoten Archipelago in northern Norway. A characteristic of this period was an extremely high wintering density within the narrow fjords. From 2002 to 2008, the herring moved offshore and are currently wintering in the area north of Vesterålen in northern Norway, but now in lower mean concentrations than during the fjordwintering period. In general, the herring distribution and migrations are extremely plastic, and large variations have been observed in the course of the past 60 years (Holst et al. 2002).

Materials and methods

Screening procedure and database

We used data from commercial purse seine catches and scientific trawl acoustic surveys between 1992 and 2008 from the Norwegian and Barents seas. The commercial catches were collected routinely from the commercial fisheries as input to the compilation of the length- and age-stratified catch statistics needed for stock assessments carried out under the auspices of the International Council for the Exploration of the Sea (ICES). The scientific trawl samples were taken by Institute of Marine Research vessels during trawl acoustic stock estimation surveys throughout the year. The same procedure was used to analyse both the commercial and scientific samples. For each sample, date, location, as well as total catch and the number of individual fish screened were registered. For each fish, age from scale readings, body and gonad weights (g), and length (cm) were recorded. The herring samples were also screened macroscopically for *Ichthyophonus hoferi*, allowing light and severe infections to be distinguished. The macroscopic sampling may underestimate the "true" infection level, because subclinical individuals, i.e., infected fish that do not show symptoms, cannot be detected (Holst 1994).

Data set levels

Because the long-term data set was highly biased towards the gear type used, the catch size, the age class of the fish, the area where the samples were taken, as well as year and month of sampling, we split the full data set into several subsets (levels) for our analyses (Table 1). Prevalence, i.e., the ratio of infected individuals in a sample, is higher in trawl catches than in purse seine catches, which is an artefact of the target school characteristic, mainly size (Kvalsvik and Skagen 1995; Holst 1996; Jovani and Tella 2006). Mean catch sizes in trawls were about 13 000 and in purse seine 660 000 fish, respectively. Because purse seine fishing was only concentrated along the coast, which does not allow for the analysis of the relation between herring migration on the disease prevalence patterns, we concentrated our analyses on trawl catches. This first level forms the data basis for the analysis of individual differences between infected and noninfected fish (Level 1, Table 1). Here, the individual data on fish were aggregated by their age class (see Analyses section).

On a second level, continuing from Level 1, we further concentrated on the Norwegian Sea ecosystem and thus excluded the Barents Sea young herring from the analyses. This excluded the majority of immature fish (<4 years old) and separated the two rather different ecosystems of the Norwegian and Barents seas. We also excluded the zero age class from the Norwegian Sea data set, as these were obtained from special trawl surveys to monitor stock recruitment. Furthermore, we concentrated on the population or schooling prevalence in the herring stock excluding small catches < 100 fish, finally resulting in 1385 samples for the analysis of the spatio-temporal development of disease (Level 2, Table 1, Fig. 1). Because infected fish display swimming disability, they are separated from the main school and are overabundant in small catches (Holst 1996),

[3] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

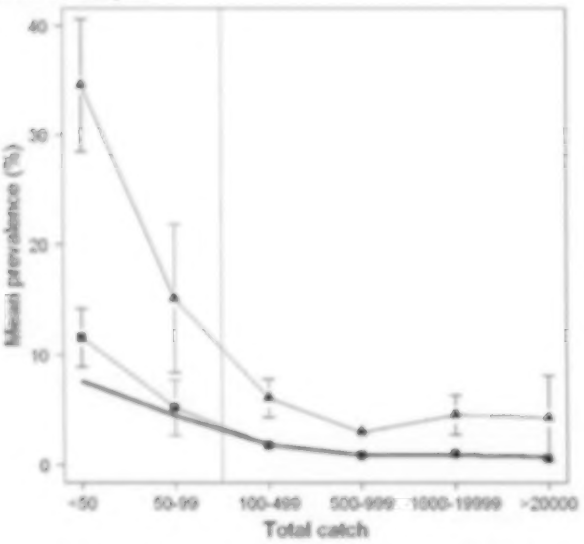
Table 1. Description of the data sets used for different levels of analysis.

Description	Level of analysis	Marine ecosystem*	Gear types used	N_{tot}	N_{inf} (light, severe)	Mean prevalence (%)
Level 0	Full data set	NA, BS	Trawl and purse seine catches	218 228	2 774 (1311, 1463)	1.3
Level 1	Individual analyses	NA, BS	Trawl catches only	147 455	2 440 (1110, 1330)	1.7
Level 2	Spatio-temporal prevalence analyses	NA	Trawl catches ≥ 100 individuals considering only age classes > 0	95 200	1 111 (572, 539)	1.2

Note: Data were taken from 1 January 1992 to 18 September 2008. N_{tot} gives the total number of individual fish sampled, of which N_{inf} were infected fish (see also Supplemental Appendix Fig. S2 (available on the journal Web site: http://cjfas.nrc.ca) regarding the number of individually screened fish at the different database levels).

*NA, North Atlantic; BS, Barents Sea.

Fig. 1. Effect of trawl catch size on *Ichthyophonus* prevalence. Mean prevalence per trawl catch size, based on the pooled data set, is show by a thick solid line. Mean prevalence per catch size with 95% confidence intervals (error bars), based on trawl catch samples taken, including those trawl catches where prevalence was 0 (i.e., where no infected fish were found), is shown by squares. Mean prevalence in positive samples (i.e., only considering samples where infected fish were found), is shown by triangles. Catches with sizes < 100 individual fish (vertical dotted line) were excluded from the analyses.



thus biasing the population prevalence level. In these small samples, the prevalence can be up to 100% (Kocan and Hershberger 2006). If we exclude the small catches, the "population prevalence" that we define in this study averages around 5% (Fig. 1), although in larger catches the prevalence is even lower. On the other hand, infected fish in small trawl catches are still part of the whole population. Furthermore, the "true" infection prevalence is probably much higher, because of detection difficulties (see above), counterbalancing the fact that we exclude a part of the population.

Variables investigated

We use infection prevalence as our measurement endpoint or dependent variable. The basic unit is prevalence per sample taken, but we also analysed the data by age or year class, space, or time units (year and month). We only used units where ≥10 data points were available per infected age or year class. We hypothesize that the effect of the disease is physiologically visible in individual fish and use body weight, gonad weight, as well as body condition K (cubic length–weight ratio; Ricker 1975; Nash et al. 2006) to describe differences in infected and noninfected fish. To detect effects of seasonality and migration on the population level, we used the wintering biomass in the fjords from 1988 to 2005 (FJORDB) (Orellana 2006; International Council for the Exploration of the Sea 2007) as an indicator of fish density in the wintering areas. Because the NSSH changed their wintering pattern from an extremely high density in the Norwegian Vestfjord system to a lower density by wintering in the open sea after 2002, we would expect a decrease in prevalence with a decrease in FJORDB over the years. Within a year, we would expect a peak in prevalence in the winter months, when fish density is highest. A source of recurrent outbreaks might be via feeding on a disease reservoir (Brunner et al. 2004). If seasonality would influence disease dynamics by feeding on a reservoir, we would expect prevalence to peak in the summer months within a year, and it could be related to zooplankton abundance. To this end, we used spatio-temporal data on zooplankton abundance (ZOOB) in May for 1995 to 2005 (Olsen et al. 2007; Ellertsen and Melle 2008). To test for environmental effects on disease prevalence, we used herring condition (K) on an annual level (Ellertsen and Melle 2008), which indicates the environmental and feeding conditions during the year for individual fish. Water temperature (TEMP) and salinity (SAL) (Olsen et al. 2007) in May were used as additional external abiotic variables. Herring recruits at age 1 (REC1) (International Council for the Exploration of the Sea 2007) was also used as an annual variable that is linked to environmental conditions such as sea water temperature (Ottersen and Loeng 2000; Toresen and Ostvedt 2000; Fiksen and Slotte 2002), i.e., a relationship between REC1 and prevalence would show the effect of several factors coming into play in the previous year. On the other hand, recruitment can be seen as a system-immanent variable, i.e., a variable that drives the host–disease system without any additional explanation. That means simply by changing the birth rate and adding susceptible fish via recruitment to the system, we would be able to obtain a recurrent epizootic pattern (Earn et al. 2000).

Analyses

Database level 1: age and year class analyses

All statistical analyses were performed in R 2.10.1 (http://www.r-project.org/) and an overview is provided (Table 2). The year class of each individual was calculated as the differ-

ence between the year in which the fish was sampled and its estimated age. We used analysis of covariance (ANCOVA) to test for differences in gonad weight (ω_G) in infected (light and severe infections pooled) and noninfected fish, controlling for their body weight (ω_B) and age class. For this purpose, ω_G and ω_B were transformed logarithmically. We also assessed individual differences per age class in infected and noninfected fish for the two variables condition K and reproductive performance (gonado-somatic index, GSI) with a t test. Differences in the prevalence pattern for age and year classes were assessed by linear regression.

Database level 2: spatio-temporal analyses of prevalence

Seasonal disease prevalence patterns (monthly analyses)

Prevalence was analysed monthly to test for seasonal peaks. The data were both pooled for all individuals as well as aggregated by sample. We used a generalized linear model (GLM, with binomial error distribution and logit link) with month as categorical variable and January as basis to test for significant differences in the prevalence, i.e., the proportion of infected fish in all samples, as well as the Wilcoxon test comparing the medians in the peak wintering month of November to the summer months of May–August in positive samples. We also tested for whether the total catch in positive samples influenced the monthly prevalence, using the Wilcoxon test. The monthly distance lag of infected behind noninfected fish in the migration pattern was calculated as the Euclidean distance of the location centres of infected and noninfected fish per month. Monthly location centres of infected and noninfected fish were calculated as the mean position (latitude and longitude) of the two groups.

Spatio-temporal disease prevalence patterns (grid-based analyses)

We first tested for differences in the prevalence per sample, depending on space (LAT, LON) and time (YEAR, MONTH) using ANOVA. We then used a raster with a grid size of 1° to aggregate spatial information (mean per grid cell) on prevalence ($PREV_{grid}$), TEMP, SAL, and zooplankton biomass (logarithmic scale) ($ZOOB_{log}$) from annual surveys in May for 1995–2005 (Olsen et al. 2007). We also calculated the prevalence in the feeding months from May to August ($PREV_{feed}$) in those years. We used Pearson's product–moment correlation coefficient r to evaluate correlations between prevalence in the feeding months and the three variables. We then used generalized additive models (GAMs, procedure gam in library gam with binomial error distribution), and the presence (prevalence > 0) or absence (prevalence = 0) of the disease per grid cell as binary dependent variable to test for nonparametric effects of our independent variables TEMP, SAL, and $ZOOB_{log}$ fitting smoothing splines with three degrees of freedom. We also included the variable YEAR to account for heterogeneity within years. Nonlinear variables were turned into suitable parametric terms before being entered into a generalized linear mixed model with YEAR as random effect (binomial GLMM using procedure glmer). We used a stepwise backward algorithm based on Akaike's information criterion (AIC) to select the final variables to describe the difference in the presence or absence of infected fish, based on the three variables. We use ΔAIC of the selected single variable model to the intercept only model as a description of the effect size of the finally selected variables.

Annual disease prevalence patterns

Annual time series of the dependent variables K, FJORDB, REC1, ZOOB, TEMP, SAL (see Variables investigated section) were correlated (Pearson's r) with mean annual prevalence ($PREV_{year}$). For longer time series (REC1), we used a moving window approach, shifting the time series backwards to detect any events, like strong recruitment, in previous years that might have substantially affected the prevalence pattern. The shorter time series of the other variables were shifted backwards by 1 year. We used the 100% minimum convex polygon to delineate the area sampled per year in contrast with the area infected per year to determine the spatial dispersion ability of the disease.

Results

Level 1: age and year class analyses

There were clear individual differences between infected and noninfected fish. Infected fish in the trawl catches were significantly lighter than noninfected fish in age classes older than 3 (lower condition K, $p < 0.01$, Fig. 2*a*), with a significant and slightly rising trend line in the condition difference with increasing age ($R^2_{adj} = 0.35$ in age classes 1–12). Gonads were significantly lighter in infected fish ($p < 0.001$ ANCOVA and GSI; Fig. 2*b* and Supplemental Appendix Fig. S3[3]), and differences were significant in all age classes except age class 11, where the number of observations was very low ($n = 21$ infected fish). The increase in difference with age was expressed increasingly in age classes 4–6, but levelled off in older age classes. In both measures K and GSI, severely infected fish were even more affected in condition and reproductive capacity than lightly infected fish (Figs. 2*a* and 2*b*). At the population level, prevalence per age and year class, respectively, also showed a significant trend, with older fish displaying a slightly higher prevalence (see Figs. 2*c* and 2*d* for significance levels).

Level 2: Spatio-temporal analyses of prevalence

Seasonal disease prevalence patterns (monthly analyses)

The disease prevalence per sample displayed a bimodal seasonality pattern, with peaks in summer (June) and winter (November) (Fig. 3*a*). There were significant differences in the monthly prevalences (Kruskal–Wallis, $p < 0.001$). The GLM for all samples revealed significantly higher prevalences ($p < 0.001$) during the summer months of May–August and in November, compared with January. December and February displayed significantly lower prevalences ($p < 0.01$). Of the positive trawl samples (light grey boxes in Fig. 3*a*), only June showed higher median prevalences ($p < 0.005$) compared with November. Light infections peaked in June, whereas severe infections had peaks in June, August, and November (Fig. 3*b*). However, we also found significant differences in the total catches per month (Kruskal–Wallis, $p < 0.05$, Fig. 3*c*), which may influence the monthly prevalence level. That is, in the winter months, the catch sizes were larger and larger catch sizes were related to lower prevalence levels (Fig. 1). That means we can expect preva-

Table 2. Overview over statistical data analyses on the different fishery data sets (Level 1 or 2) and spatio-temporal scales.

Unit or spatio-temporal scale	Measurement endpoint	Statistics used
Level 1		
Individual fish, pooled for time, space, and sample	*K* and GSI in the different age classes	*t* test and regression through residuals
	Body length and weight as well as gonad weight in the different age classes	ANCOVA
	Prevalence per age and year class	Linear regression
Level 2		
Spatio-temporal level per sample	Prevalence vs. LAT, LON, YEAR, MONTH	ANOVA
Spatio-temporal level per grid cell and year (1995–2005)	Prevalence vs. TEMP, SAL, ZOOB	Pearson's *r*
Spatio-temporal level per grid cell and year (1995–2005)	Presence–absence of infected fish vs. TEMP, SAL, ZOOB	GAM and GLMM
Seasonal (monthly) level aggregated per sample, pooled for years and space	Prevalence per month	GLM and Wilcoxon test for differences in summer and winter prevalence
Spatio-seasonal (monthly), pooled for years, space, and sample	Location centres (LAT, LON)	(Visual)
Annual level, pooled for space and sample	Population prevalence vs. *K*, FJORDB, REC1, ZOOB, TEMP, SAL	Pearson's *r*

Note: Each measurement endpoint was tested for differences of infected vs. noninfected fish (Level 1) or differences in prevalence (Level 2). A sample corresponds to a catch. For details of variable names, see main text.

lence levels in the winter months as being even higher. When this effect was corrected for by considering only small catches between 100 and 1000 fish (Fig. 3*d*), there was a peak in August, coinciding with the peak in the severely infected fish (Fig. 3*b*) and supporting the finding that the small catches contain those fish that could not keep up with their school during migration. Although the infected individuals follow the migration of the healthy fish (Supplemental Appendix Fig. S4[3]), the infected fish lagged behind most of the others (difference in location centres) in April and July, not reaching the feeding grounds in the most distant areas of the Norwegian Sea (Fig. 4*b*) and thus forming a spatial "infectious belt" that noninfected adult fish leave behind and come again into contact with on their migration route back to the wintering areas.

Spatio-temporal disease prevalence patterns (grid-based analyses)

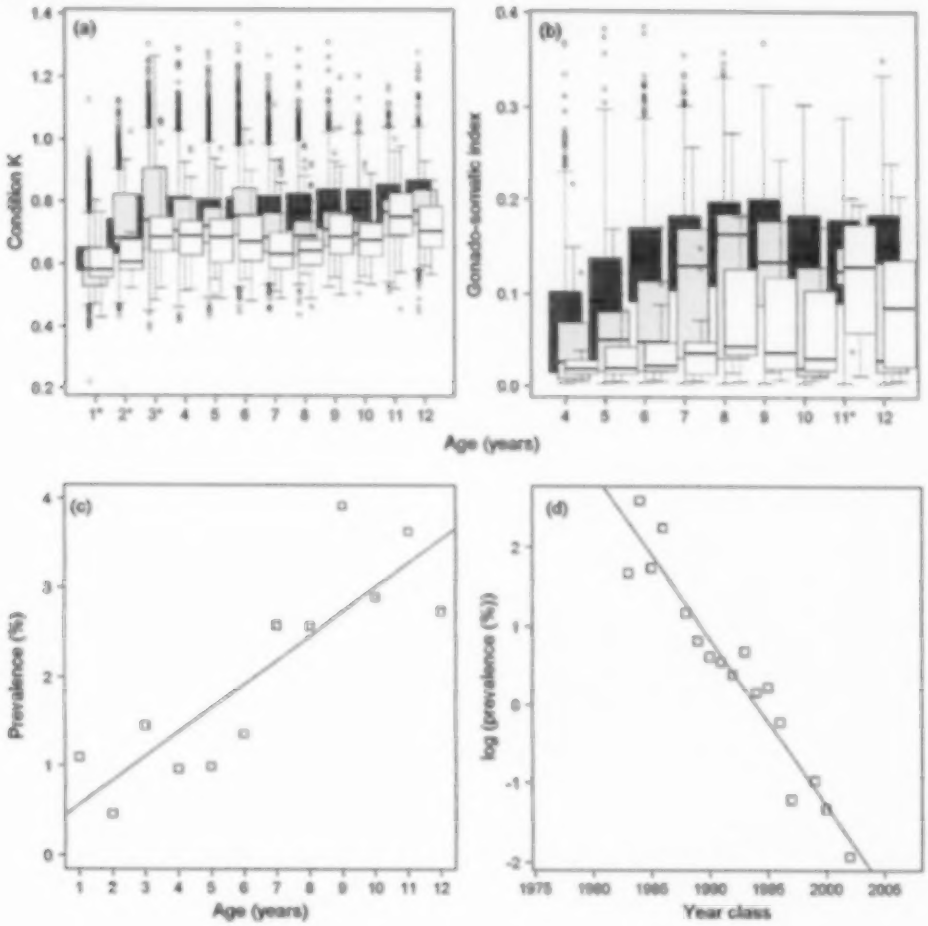
The highest prevalence in space (year data pooled) was recorded in the feeding grounds in the Norwegian Sea and along the border with the Barents Sea (Fig. 4*a*), but prevalence varied across space (ANOVA, latitudinal gradient, $F = 4$, $p \leq 0.05$) and over time (year, $F = 35$, $p \leq 0.001$). Prevalence in the winter months (November to February) was more or less evenly distributed amongst the older age classes along the coast, with a maximum prevalence of ~7%, but the youngest age classes within the fjords were less affected (Fig. 4*c*, Pearsons's $r = 0.27$, $p = 0.02$ for spatial age–prevalence relationship). In the summer months prevalence peaks of up to 30% were reached, mostly distributed among the medium ages (Pearsons's $r = 0.17$, $p = 0.018$ for spatial age–prevalence relationship) building the "infectious belt" and thereby most likely infecting the medium-aged fish.

ZOOB in May per grid cell for the years 1995–2005 did not show a significant correlation with prevalence in the feeding months (May to August, $p > 0.5$), nor did SAL ($p > 0.1$), but there was a weak positive relationship between prevalence and TEMP (Pearson's $r = 0.18$, $p < 0.05$), with most positive prevalence measures found between 4 and 7 °C. No infection was found in temperatures < 4 °C. However, TEMP and SAL themselves were correlated ($p > 0.001$, $r = 0.46$). The GAM analysis revealed significant nonparametric effects for TEMP ($p < 0.05$) and year ($p < 0.01$). Therefore, TEMP was entered as polynomial term into the GLMM, and the year was included as random effect. Eighty-two variables were entered into the GLMM. Based on the AIC selection algorithm, the best model contained the variables SAL and ZOOB. However, the difference in AIC was low ($\Delta AIC \sim 2$), indicating only poor ability in discriminating presences, i.e., infected fish, from absences.

Annual disease prevalence patterns

The disease prevalence patterns over time revealed a major peak at the beginning of the epizootic, with a mean population prevalence ($PREV_{year}$) of about 9% and oscillations with decreasing prevalence peaks over time (Fig. 5*a*). Three main phases can be distinguished, i.e., the peaks in 1993 and 1999 and a small peak in 2004, but there were few data on infected individuals ($n = 12$ for year 2004, $n < 10$ for the years 2002, 2003, and years beyond 2004; Fig. 5*a*). In 2008 the disease was present at an extremely low level for the whole population (Fig. 5*a*), but stayed at about 3% in the infected catches (Fig. 5*b*). The peaks also showed a decline in the severity of the disease, i.e., the prevalence in severely infected fish is larger in the first outbreak and then declines over the years (Fig. 5*a*). Prevalence in the summer months in the time series 1992–2008 (May–August; slope = –0.003, $p < 0.001$) decreased more rapidly than in winter (November–February; slope = –0.0012, $p < 0.001$). This is partly due to the fact that the high prevalence peak in the

Fig. 2. (*a* and *b*) Medians of noninfected (dark grey) vs. lightly (light grey) and severely infected (open) age classes for condition (*K*) and gonado-somatic index (GSI). An asterisk (*) marks nonsignificant pairs (*t* test, $p > 0.01$). (*c* and *d*) Prevalence per age and year class. The year class was calculated as the difference between the year the fish was sampled and its age at sampling. There is a significant relationship between age (*c*: estimate = 0.003, $p < 0.001$, $R^2_{adj} = 0.71$) or year class (*d*: estimate = –0.2, $p < 0.001$, $R^2_{adj} = 0.91$) and prevalence. The data were collected from 1992 to 2008.

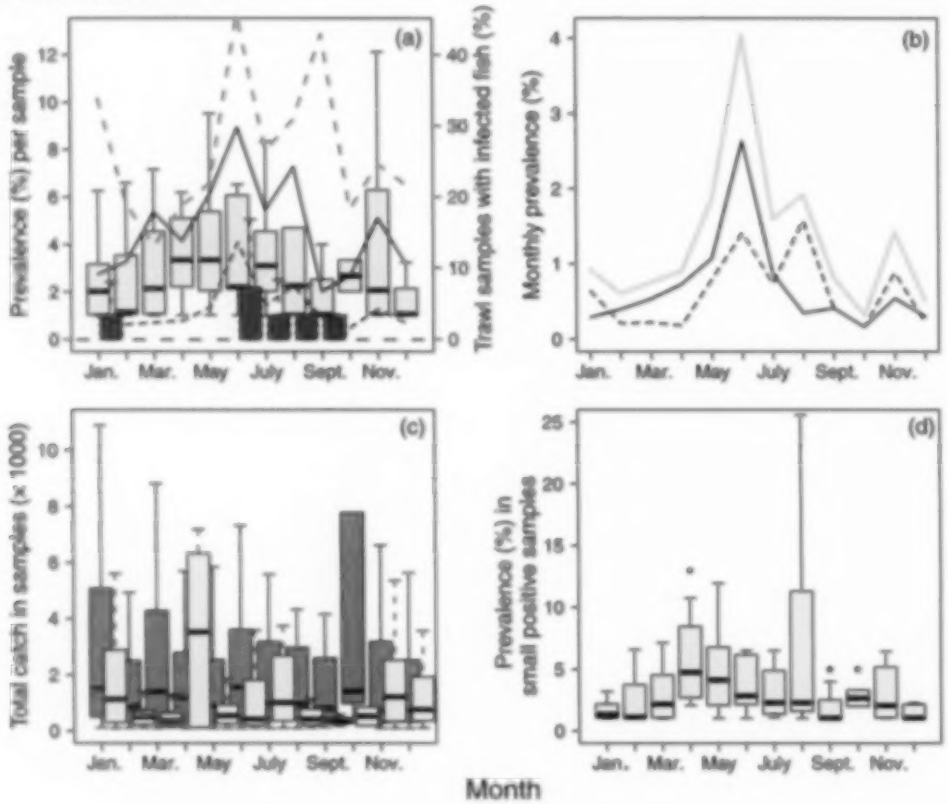


1993 data is caused by data from the winter months only (January and December; Supplemental Fig. S5[3]), i.e., there is no summer data available for the year with the first outbreak. The annual disease spread, i.e., the area infected compared with the area sampled, followed the annual prevalence pattern (Fig. 5*a*, right axis), i.e., high annual prevalence was correlated with finding infected fish distributed across a large area. The sample prevalence, i.e., the proportion of all samples taken per year vs. samples with disease-positive fish, also followed the general trend with a second peak in 1999 (1992–1993 prevalence = 80%–100%, 1999 = 40%, down to 3% in 2008, data not shown). When we correlated the annual prevalence pattern (infected individuals pooled, i.e., no distinction between light and severe infections) with the time series of the respective variables, only *K* (Pearson's $r = 0.86$) and REC1 ($r = 0.56$) were significant. ZOOB, TEMP, SAL, and FJORDB did not display a significant correlation with $PREV_{year}$ at an aggregated annual level. Within a year class and over time, *I. hoferi* prevalence followed the overall annual prevalence level (results not shown).

Discussion

Diseases in marine fish populations and their impact on populations have been relatively little studied because of many constraints regarding sampling possibilities as well as our lack of knowledge of host–pathogen systems. We present the first long-term study of an epizootic in a highly migratory fish species, which is a special challenge in spatially separating causes and effects. Although we introduce important results and analyses conducted for the first time for the *Ichthyophonus* epizootic in NSSH, we cannot clearly confirm any particular driver. Rather, several mechanisms seem to be involved in the perpetuation of the disease that we discuss below. We describe the limitations of this long-

Fig. 3. (*a*) Medians (boxplots) and means (black lines) of monthly prevalence in positive samples (solid black line and light grey boxes) and all samples (dashed black line and dark grey boxes, shifted to the right). Outliers are not shown. The grey dotted line refers to the right axis. (*b*) Seasonal prevalence pattern for lightly (solid black line) and severely (dotted line) infected individuals as well as overall prevalence (grey line). Data were pooled by month. (*c*) Monthly fishing effort expressed as the median (boxplot) of the total catch per noninfected (dark grey) and infected sample (light grey). (*d*) Median prevalence (boxplot) in small samples (total catch 100–1000 fish). Months with asterisks (*) had less than 10 observations.



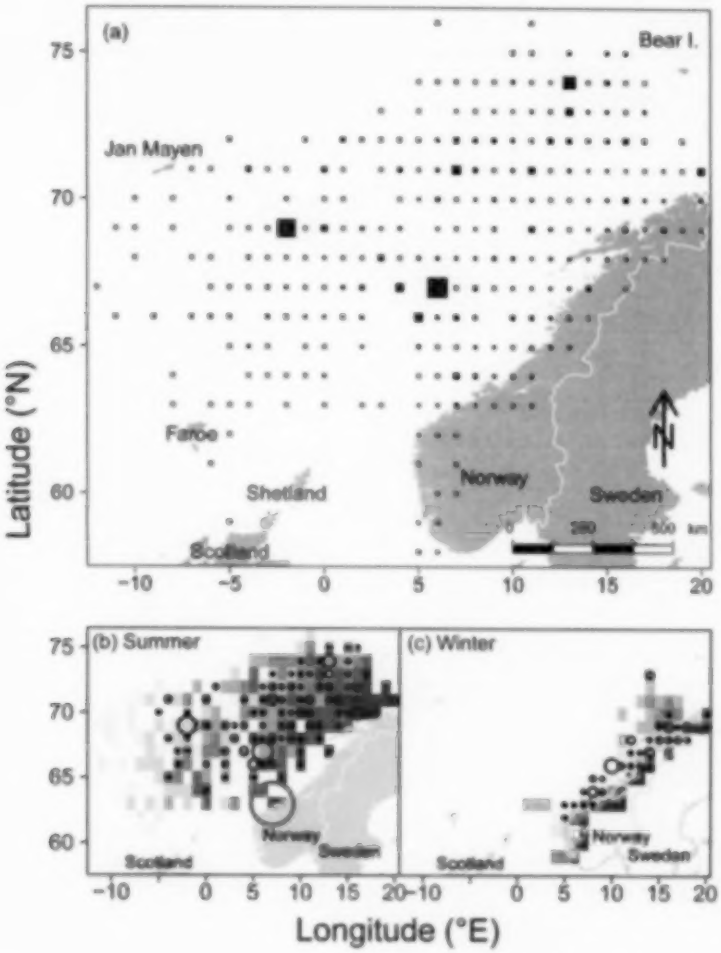
term data set for understanding marine disease dynamics and offer recommendations for future monitoring to advance our understanding of the ecology of marine diseases.

Individual differences

The disease significantly reduced the body condition and reproductive capacity of infected fish (see also Holst et al. 1997, based partly on the same data set). Differences in body condition K between infected and noninfected fish, for example, start to be significant when they are >3 years old, i.e., when they enter the Norwegian Sea. This age-related difference in K may stem from the fact that the young fish put all their energy into somatic growth for survival, i.e., those with lower K have already died and therefore did not appear in the catches. Differences in the GSI measure between infected and noninfected individuals were pronounced at maturation between the ages of 4 and 6, resulting in older fish having significantly reduced reproductive capacity. Seen from a life-history perspective in allocating resources into growth, reproduction, or energy storage (Jørgensen et al. 2006), the infected fish that had survived until being sampled may show a trade-off; the diseased fish may have survived at the expense of gonad growth. The loss in spawning capacity in addition to disease mortality may substantially affect herring population dynamics (Marty et al. 2003).

The observed age-related increase in prevalence has also been reported for Pacific herring (Marty et al. 1998; Hershberger et al. 2002). It may indicate that there is no acquired immunity and that older individuals can accumulate, i.e., live longer with, the pathogen. The low prevalence in younger fish in the samples may simply be due to the fact that they die off sooner and have disappeared before being sampled, as could be deducted from the findings for K (see above). The possibility that younger fish are more sensitive to infection by *I. hoferi* and that they become temporarily less susceptible to the lethal effects of the pathogen with increased age is supported by McVicar and McLay (1985). On the other hand, it could also be a difference in exposure due to differences in the feeding and migratory pattern as hypothesized in salmonids (Kocan and Hershberger 2006). Seasonality and spatial heterogeneity have been recognized as important factors that contribute to differences in pathogen exposure (Wilson et al. 2001). We discuss this in the following section.

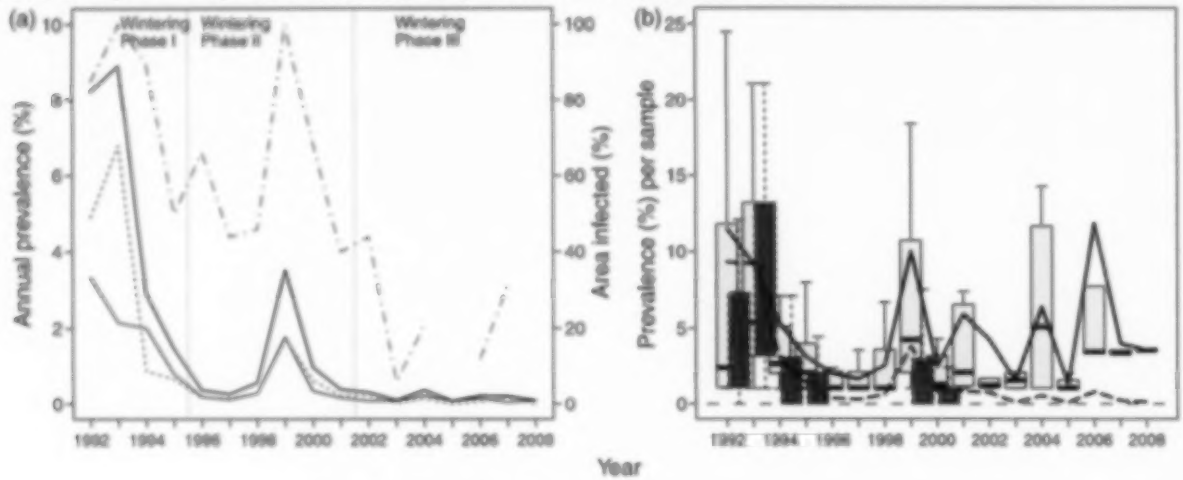
Fig. 4. (*a*) Mean prevalence per sample and grid cell. Rectangles show increasing prevalence to a maximum of 30%. Horizontal lines indicate samples taken, but no infected fish found. Note: points lying on the Norwegian mainland are artefacts from aggregating spatial data on a 1° grid. These points were data from within the fjords that stretch far into the mainland. Because spatial units are given in decimal degrees, the scale bar is only valid for the region where it is plotted. (*b* and *c*) Prevalence (black circles) and mean age gradient (red (young: age 1) to yellow (age 12)) for the summer (May–August) (*b*) and winter (November–February) (*c*) months. The large grey circle in (*b*) with prevalence up to 75% is an outlier. It stems from a single scientific survey conducted at the beginning of the outbreak in 1992 in the youngest age class.



Spatio-temporal patterns

Several mechanisms must interact to produce the epizootic pattern found. On a long time scale, prevalence decreases, with peaks in prevalence displaying within a 5- to 6-year period, together with strong annual seasonality. Similar seasonal disease prevalence patterns had been observed in the eastern North Atlantic off the Swedish coast (Rahimian and Thulin 1996). Generally speaking, seasonality is regarded as "force majeure" in natural systems, triggering recurrent epizootics by changing, for example, the contact rate between individuals (Hosseini et al. 2004; Altizer et al. 2006; Stone et al. 2007). There was an effect of seasonal changes in stock density visible in the seasonal prevalence peak in the winter months, increasing the contact rate or exposure to spores, which decline more slowly over the years than the prevalence in summer. However, there was no correlation with the size of the wintering stock biomass. It is worth mentioning that the characteristics of an increasing spawning stock biomass at the beginning of the epizootic as well as a high wintering density in a fjord system also applied to the Icelandic stock in which an *Ichthyophonus* outbreak occurred in autumn 2008 (Oskarsson and Palsson 2009). This parallel suggests that density has at least some effect in the disease transmission process. In 1990, the whole NSSH stock changed both its wintering habits by wintering in extreme densities in the Lofoten area and its summer feeding habits in 1991, when it fed over large parts of the Norwegian Sea, mainly on the copepod *Calanus finmarchicus* (Holst et al. 2004). This, together with the summer peaks in seasonal prevalence and thus spatial prevalence peaks in the feeding areas, at least in a local spatial context, may support the premise that infected food items

Fig. 5. (*a*) Mean annual prevalence of lesions associated with *Ichthyophonus*: mild infection (thin solid line), severe infection (dashed line), and all cases combined (thick solid line). The grey line (dot-dashed, right axis) shows the percent area infected of the total area sampled. In 2005 and 2008, there were not enough data points of infected fish to calculate the minimum convex polygon. The grey vertical lines delineate the three phases of wintering: extremely dense between 1990 and 1995 in the inner fjord system, very dense between 1996 and 2001 in the complete fjord system, and then thinning out while wintering in the open sea. (*b*) Medians (boxplots) and means (lines) of monthly prevalence in positive samples (solid line and light grey boxes) and all samples (dashed line and dark grey boxes, shifted to the right).



serve as a reservoir of disease. Torgersen et al. (2002) and Jepps (1937) found *Calanus* species, the main zooplankton food source of NSSH, infected with what was presumably a pathogen of the genus *Ichthyophonus*. But *Calanus* has not yet been systematically checked for diseases, which opens up new paths for research. However, there was no strong significant effect of zooplankton abundance on prevalence or disease presence, and the differences in the seasonal peaks could be artefacts indirectly related to environmental conditions. The average water temperature is higher in the Norwegian Sea than in the Barents Sea where the recruiting stock is up to the age of 3, and this has been shown to positively affect disease prevalence, although infections were found in the Barents Sea as well (database level 0, see Materials and methods section). The higher prevalence in the summer months than in winter could be due to better development of the pathogen in warmer water. Positive correlations between water temperature and *Ichthyophonus* infections have also been reported for other fish species, like the Buffalo sculpin (*Enophrys bison*) and rainbow trout (*Oncorhynchus mykiss*) (Okamoto et al. 1987; Halpenny et al. 2002). Spatially, the highest prevalence in the Norwegian Sea was found along its border with the Barents Sea and western-central regions; both areas are characterized by warm water currents (cf. figure 1 in Olsen et al. 2007). But again, this might be a spurious correlation. The area along the border to the Barents Sea is the herring return route to the coast. The spatial aggregation of prevalence there may be an artefact of seasonality in migration, where the infected older fish unable to migrate to the furthest feeding ground aggregate, thereby forming an infectious belt with the potential for disease transfer to cohabiting medium-aged fish. Another important result addresses at least an indirect effect of environmental conditions in *Ichthyophonus* dynamics. In this case, strong recruitment in a certain year correlated significantly with body condition and prevalence peaks in the following year. Recruitment and body condition are related to environmental conditions (Ottersen and Loeng 2000; Toresen and Ostvedt 2000; Fiksen and Slotte 2002), which may explain the relationship with *Ichthyophonus* prevalence, at least on the visible macroscopic scale. Clinical experiments by Kocan et al. (1999) have shown that prevalence increases with age with no associated increase in mortality, leading to the conclusion that fish in good condition or with a well-developed immune system can stand higher loads of the pathogen. Similarly, the bimodal seasonal pattern of infection prevalence could have resulted from environmental factors exacerbating subclinical (undetected) infections to overt disease. The recurrent peaks of infection in the epizootic every 5 or 6 years could stem from susceptible strong year classes maturing and entering the Norwegian Sea for their first spawning close to the wintering areas. The input of susceptible maturing herring, especially of strong year classes, could have fuelled new epizootic waves (Earn et al. 2000). Microparasites usually cause epizootics when waves of infection pass through the populations, after which the pathogen disappears as a result, for example, of disease-induced death (Tompkins et al. 2001). Previous *Ichthyophonus* epizootics have been associated with acute mass mortalities of short duration (<6 months; Sindermann 1956). Although there have been several pulses in recruitment in the past few years, the prevalence decreased, more or less ending the epizootic in 2008. But going hand in hand with a decline of the epizootic are three more facts: zooplankton abundance in the central Norwegian Sea has been low for several years (International Council for the Exploration of the Sea 2007), which also affects the overall herring body condition, and stock density in the wintering areas has decreased in three major steps, coinciding with the decreasing peaks in prevalence. A third process that must be incorporated in a more

integrated approach to understanding disease dynamics in ecosystems is the role of predators (Tjelmeland and Lindstrom 2005). An increase in selective predation pressure on the infected fish — in contrast with a fishery that selects for rather large schools with low prevalence — may have led to a thinning out of the pathogen. Indeed, many diseased herring were found in the stomachs of cod (60%–80% of the stomach contents) during the first major epizootic wave (J.C. Holst, personal observation). In summary, we conclude that the interplay of several circumstances and plausible mechanisms may have led to the severity and long duration of the outbreak with changes in host behavioural (wintering, schooling) and ecological (feeding migration, predation pressure, environmental conditions) patterns difficult to disentangle.

Recommendations

In spite of the existence of an enormous body of knowledge about the host NSSH and the huge sampling efforts that have led to this unique long-term data set, we must conclude that we cannot be sure about what we are really seeing because we still deal with a correlational study. For example, the positive association between water temperature and prevalence — be it seasonally in summer or locally in space — may simply be due to the fact that the pathogen develops better and grows faster in warm water. On the other hand, the lower prevalence peak in winter compared with summer may indicate increased mortality due to worse conditions for the herring, be these a huge exposure to spores due to an extreme winter density or a lower body condition due to the absence of feeding or unfavourable winter environmental conditions. Much more knowledge is therefore needed about the pathogen side, and we recommend the following studies. (*i*) Microscopic as well as macroscopic sampling is urged, as this would offer a higher probability of detection of the infection (Holst 1994; Rahimian and Thulin 1996). (*ii*) As in other impact assessment studies in terrestrial ecology that are designed to yield statistical inferential strength, e.g., before–after control–impact (BACI) studies (Roedenbeck et al. 2007), we also need long-term knowledge on the situation before the outbreak, i.e., microscopic sampling or serological tests that tell us about previous other infections or co-infections, which has been shown to be important in Pacific herring (Marty et al. 1998). This is in agreement with the priorities for investigating marine disease dynamics set by Kim et al. (2005). (*iii*) Genotyping is also helpful in studying the global spread pattern or the origin of the pathogen (Rachowicz et al. 2005), as has been done, for example, for *Ichthyophonus* in the Pacific Ocean (Criscione et al. 2002). Finally, setting up an appropriate monitoring scheme to better understand disease dynamics in the ocean will contribute to further progress in marine disease ecology.

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Modeling cadmium uptake from water and food by the freshwater bivalve *Pyganodon grandis*

Sophie Cooper, Landis Hare, and Peter G.C. Campbell

Abstract: For this study, the unionid bivalve *Pyganodon grandis* was either exposed to dissolved cadmium (Cd) or fed Cd-contaminated algae (*Pseudokirchneriella subcapitata*) during short-term laboratory experiments. Cadmium accumulated largely in the digestive gland after a dietary exposure, or in the gills after an aqueous exposure; in these latter experiments, Cd accumulation from the dissolved phase increased as bivalve filtration rates increased. The results of these uptake experiments were used to parameterize a biodynamic model, which was then used to estimate the relative importance of water and food as sources of Cd for this bivalve, and to predict steady state Cd concentrations in the gills and digestive gland of native bivalves. In comparisons between the simulations and data obtained from earlier field studies on *P. grandis*, the model adequately predicted Cd concentrations in *P. grandis* gills, except in Ca-rich lakes, whereas it tended to overestimate Cd concentrations in the digestive gland. The field simulations indicate that water is the main source of Cd for both the gills (relative importance water:food::99:1) and the digestive gland (water:food::80:20). These results will facilitate the interpretation of spatial and temporal variations in Cd concentrations in free-living *P. grandis*, which is a promising metal biomonitor.

Résumé : Des bivalves unionidés *Pyganodon grandis* ont été ou bien exposés à du cadmium (Cd) dissous ou alors nourris d'algues (*Pseudokirchneriella subcapitata*) contaminées au cadmium lors d'expériences de courte durée en laboratoire. Le cadmium s'accumule en grande partie dans la glande digestive après une exposition alimentaire ou dans les branchies après une exposition dans l'eau; dans ces dernières expériences, l'accumulation de Cd à partir de la phase aqueuse croît en fonction de l'augmentation des taux de filtration du bivalve. Les résultats de ces expériences d'accumulation ont servi à déterminer les paramètres d'un modèle biodynamique qui a ensuite permis d'estimer les importances relatives de l'eau et de la nourriture comme sources de Cd chez ce bivalve et à prédire les concentrations de Cd à l'état d'équilibre dans la glande digestive et les branchies des bivalves indigènes. Lors de comparaisons entre les simulations et des données obtenues d'études antérieures en nature sur *Pyganodon grandis*, le modèle prédit de manière adéquate les concentrations de Cd dans les branchies, excepté dans les lacs riches en Ca, alors qu'il a tendance à surestimer les concentrations de Cd dans la glande digestive. Les simulations en nature indiquent que l'eau est la source principale de Cd, tant dans les branchies (importances relatives eau : nourriture :: 99:1) que dans la glande digestive (eau : nourriture :: 80:20). Ces résultats vont faciliter l'interprétation des variations spatiales et temporelles des concentrations de Cd en nature chez *P. grandis*, un indicateur biologique des métaux prometteur.

[Traduit par la Rédaction]

Introduction

The effects of metal contamination on aquatic ecosystems have received considerable attention over the past 30 years. Monitoring programs using aquatic invertebrates, such as bivalves, have been developed in many countries to investigate the risk such contamination may pose for aquatic organisms and ecosystems (Jernelov 1996). Early risk assessment or biomonitoring programs were based on the assumption that dissolved metals were the main source of contamination for filter-feeding invertebrates (Schlekat et al. 2001). However, bivalves are exposed to metals from both the particulate and dissolved phases, and subsequent studies have revealed that metal uptake from food can be an important source of metals for these animals (Luoma et al. 1992; Wang et al. 1996; Reinfelder et al. 1998). Given the potential importance of dietary metal exposures, there is a need to quantify the relationship between ambient metal concentrations and bivalve metal concentrations and, in particular, to determine the relative importance of food and water as sources of metals for these animals.

Previous efforts to model metal accumulation in marine and freshwater bivalves, performed using regression analyses, determined that metal concentrations in soft tissues can vary as a function of the aqueous concentration of the free metal ion (as computed for Cd by Tessier et al. (1993) for

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Pyganodon grandis) or depend on metal accumulation from food (particularly for Cd, Cu, Hg, and Zn; Thomann et al. (1995) for *Mytilus edulis*). However, these studies did not directly quantify the uptake of metals from food and water into the bivalves. This goal can be achieved using biodynamic models (Roditi et al. 2000; Luoma and Rainbow 2005). Such models require information on uptake rates of contaminants from food and water pathways, as well as contaminant efflux rates and animal growth rates. They are inherently flexible and applicable to diverse environments under variable conditions (Landrum et al. 1992; Schlekat et al. 2001).

Previous research has identified *P. grandis* as a potential sentinel organism to assess spatial and temporal trends in bioavailable Cd concentrations (Couillard et al. 1995; Giguère et al. 2003; Bonneris et al. 2005). This species is widely distributed in freshwater lakes and rivers across North America and could thus be suitable for broad regional comparisons, especially in freshwater systems that have been perturbed by industrial and mining activities. Although consideration has been given to using *P. grandis* as a biosentinel, no previous study has quantified the relative importance of various metal uptake pathways for *P. grandis*, or determined the kinetic parameters (e.g., filtration rates, ingestion rates and assimilation efficiencies) needed for the development of a metal bioaccumulation model for this bivalve. We therefore conducted a series of laboratory experiments to determine the absorption efficiency of dissolved Cd and the assimilation efficiency of diet-borne Cd, as well as bivalve filtration and ingestion rates. Based on the parameters obtained in the laboratory, we parameterized a kinetic model of Cd bioaccumulation for *P. grandis* and compared the results predicted by the model with those obtained from earlier field studies. We focused our study on the trace metal Cd because it is reported to be the cause of toxic effects on animals living in lakes situated in the vicinity of a major Canadian metal smelter (the Horne smelter in Rouyn-Noranda, Quebec, Canada) (Borgmann et al. 2004; Campbell et al. 2008; Pierron et al. 2009).

Materials and methods

Bivalve collection and maintenance

Specimens of *P. grandis*, ranging in size from 5 to 9 cm, were collected from a lake having low trace-metal concentrations (Lake Opasatica, located upwind from the Horne smelter). Bivalves were transported to the central laboratory, in Quebec City, in coolers filled with aerated lake water. Lake sediments were also collected and transported to the laboratory. A stock culture was set up in a cold room maintained at 15 °C. Bivalves were placed in 55 L plastic containers filled with 50 L of dechlorinated tap water and 5 cm of unsieved lake sediments. Fluorescent lamps and timers were used to maintain a 12 h (day) – 12 h (night) photoperiod. The water in the containers was renewed weekly by removing two-thirds of the water and refilling the containers with fresh dechlorinated tap water. Bivalves were fed daily with a 50–50 (cells:cells) mixture of the green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), grown under laboratory conditions, and a commercial food composed of marine algae (Phytoplex, Kent Marine, Acworth, Georgia, USA; containing *Nanochloropsis* sp., *Tetraselmis* sp,. and *Isochrysis* sp.). Bivalves were allowed to acclimate for one month before being used in experiments.

Table 1. Chemical composition of the synthetic medium (medium A) used during waterborne and diet-borne cadmium exposure experiments on *Pyganodon grandis*, and the simplified synthetic medium (medium B) used to expose the alga *Pseudokirchneriella subcapitata* to cadmium and to rinse the algae after aqueous Cd exposure and before experimentation with the bivalves.

Element	Medium A ($mg \cdot L^{-1}$)	Element	Medium B ($mg \cdot L^{-1}$)
Ca	26	Ca	3.4
Mg	3	Mg	3.6
K	2	Na	38.7
Na	3	SO_4	14.6
SO_4	28	NO_3	91.2
NO_3	0.17	Cl	13.5
CO_3	5		
Cl	2		

Prior to an experiment, individual bivalves were transferred into separate 2 L plastic beakers filled with synthetic medium (medium A: Table 1), the chemical properties of which resembled those of the tap water. The use of the synthetic medium-A was designed to minimize the concentration of organic matter that might bind dissolved Cd in the exposure medium and thereby reduce its uptake by the bivalves. Synthetic medium-A was prepared one week in advance and its pH was adjusted (average pH 7.1) prior to use. Bivalves were allowed to acclimate for 4–5 days in the experimental set-up. They were fed daily during the acclimation period and the medium was changed every 2 d. Bivalves were then starved for 24 h prior to an experiment. Control experiments, during which one group of bivalves was starved and another group of bivalves was fed continuously for 3 d, showed no significant difference between the average filtration rates of fed and starved bivalves (23 ± 4 $mL \cdot h^{-1} \cdot (g\ fresh\ mass)^{-1}$ for fed bivalves; 26 ± 5 $mL \cdot h^{-1} \cdot (g\ fresh\ mass)^{-1}$ for starved bivalves; ± standard error (SE); $N = 9$; Student's t test, $p > 0.05$).

Aqueous exposure to cadmium

Bivalves ($N = 54$) were exposed to several concentrations of total dissolved Cd: 0.1, 0.5, 5.0, and 20.0 $nmol \cdot L^{-1}$ [specific activity: 0.02–0.15 $MBq \cdot (\mu g\ total\ Cd)^{-1}$]. There were nine bivalves for each individual experiment; exposures at 0.5 $nmol \cdot L^{-1}$ and 5.0 $nmol \cdot L^{-1}$ were replicated [i.e., 2 × 9 bivalves). For each Cd concentration, four 6 L aquaria (3 experimental aquaria, each containing 3 bivalves + 1 control aquarium without bivalves) were filled with synthetic medium-A containing dissolved Cd. Bivalves were exposed to the dissolved Cd for three consecutive days excluding daily feeding periods, that is, after a 20 h exposure period, each bivalve was transferred from its experimental aquarium into a separate 2 L beaker filled with uncontaminated synthetic medium-A and fed with uncontaminated algae for 4 h. This procedure ensured that algae would not become contaminated with Cd during feeding. We determined the filtration rate for each bivalve during this feeding period by measuring the rate at which the algal cells were cleared from the

suspension (see below). After 4 h, the bivalves were transferred back into their experimental aquaria for another 20 h exposure period. During the feeding period, the concentration of dissolved Cd in the exposure aquaria was returned to its initial concentration prior to the bivalves being placed back into the aquaria. After 72 h, the bivalves were placed in separate beakers to depurate overnight before dissection. In total, the bivalves were exposed to dissolved Cd for 60 h during the 3 d exposure.

Dietary exposure to cadmium

This experiment consisted of two phases: (*i*) contamination of the green alga *P. subcapitata*, and (*ii*) feeding contaminated algae to *P. grandis*. Algal cells were separated from their Bristol culture medium (University of Texas (UTEX) 2009) and rinsed 3× by centrifugation (7000 rpm; 5500*g*) with a simplified, EDTA- and PO_4-free, culture medium (medium B; Table 1). The algal cells were then transferred into two 1 L Erlenmeyer flasks containing medium B to which Cd had been added [4.6 nmol·L^{-1}; specific activity 26 MBq·(μg total Cd)$^{-1}$]. This exposure medium-B was buffered with HEPES (10 mmol·L^{-1}; 1-piperazine-4-(2-hydroxyethyl)-ethanesulphonic acid) to ensure that the pH would remain at 7.5. The algal cells remained in the Cd-contaminated medium for 1 d. To reduce the sedimentation of algal cells during the exposure period, the algal suspension was stirred using a magnetic stirrer. After 1 d, 3 replicate subsamples (3 × 5 mL) were collected and used to determine the amount of Cd accumulated by the algae (see below). The remaining algal cells were then separated from the contaminated medium by centrifugation and rinsed as described above. After the initial rinsing, the algal cells were washed with Na_2EDTA (10^{-4} mol·L^{-1}) for 20 min to eliminate any Cd that had adsorbed onto their surface (Hassler et al. 2004). The algal cells were then rinsed 3× with the simplified medium-B to remove any remaining EDTA and dissolved Cd.

The three replicate samples of the exposure medium-B that were collected prior to centrifugation were filtered through two polycarbonate membranes (2 μm, Nuclepore) and rinsed first with the simplified medium B (3 × 5 mL) and then with 10^{-4} mol·L^{-1} Na_2EDTA (3 × 5 mL) to remove adsorbed ^{109}Cd. The first membrane served to collect the algae, whereas the second membrane was exposed only to dissolved ^{109}Cd and thus served as a filtration blank (accounting for any passive sorption of ^{109}Cd to the filter). Each filter was placed in a separate scintillation vial and analysed in a Gamma counter (Wallac 1480 Wizard™, Turku, Finland). The counting window for ^{109}Cd was between 16 keV and 32 keV, with a peak maximum at 22 keV. The counting period was fixed at 2000 s (33 min), which insured a minimum of 80 000 counts (average counting error of 1%). The observed radioactivity was converted to total Cd concentrations, on the basis of the known ^{109}Cd specific activity, and these concentrations in the algae were expressed in nmol Cd·g^{-1}. The mass of algae on the filter was calculated on the basis of the volume filtered (5 mL), the algal concentration per unit volume (~15 000 000 cells·mL^{-1}), and the mass of an algal cell (dry mass = 1.8×10^{-12} g·cell^{-1}). Algal density and cell dimensions (i.e., surface and diameter) were obtained with a particle counter (Beckman Multisizer™ 3 Coulter Counter, Miami, Florida, USA).

Nine bivalves were placed in separate 2 L feeding beakers filled with synthetic medium-A that contained Na_2EDTA (10^{-4} mol·L^{-1}) to capture any dissolved inorganic Cd released by the algae during the experiment. Each bivalve was given Cd-contaminated *P. subcapitata* at a density of 400 000 cells·mL^{-1}. The bivalves were allowed to feed on the contaminated algae for 4 h, and their filtration rates were measured during this period. Desorption of Cd from the algal cells was reduced by rinsing the algae with Na_2EDTA prior to feeding the bivalves. After 4 h, the bivalves were transferred into an algae-free 6 L holding tank filled with synthetic medium-A spiked with Na_2EDTA (10^{-4} mol·L^{-1}) to capture any dissolved Cd released as faeces or urine. These precautions ensured that any Cd that accumulated in *P. grandis* during the dietary exposures was truly from a dietary source and not from solution. The bivalves remained in the tanks for 20 h, after which they were transferred back into a feeding beaker that contained fresh medium-A and algae. This sequence of 4 h feeding and 20 h fasting was repeated 4×. In all, the experiment lasted 96 h, during which bivalves were exposed to Cd-contaminated algae for 16 h. The experiment was repeated once more for a total of 18 bivalves ($N = 9 \times 2$).

Once the exposure period was completed, the bivalves were transferred into feeding beakers that contained fresh medium-A spiked with Na_2EDTA (10^{-4} mol·L^{-1}) and they were allowed to depurate for 8 d. During the depuration period, bivalves were fed continuously with uncontaminated *P. subcapitata* to encourage them to purge any contaminated algae.

Organ and data analysis

After exposure to either aqueous or dietary Cd, bivalves were sacrificed and dissected. The various organs (gills, digestive gland, mantle, foot, and miscellaneous organs (including the gonads and intestines)) were separated and rinsed with EDTA (10^{-3} mol·L^{-1}) for 20 min to remove any Cd adsorbed to their surface. The organs were weighed individually, placed in a scintillation vial and analysed for ^{109}Cd using a Gamma counter (as described above). Cadmium concentrations for each organ were calculated and expressed in nmol total Cd·(g fresh mass)$^{-1}$.

The contributions of the various bivalve organs to the total Cd burden (expressed as percentages), as obtained for each Cd treatment, were transformed (arcsine) and compared using a one-way analysis of variance (ANOVA, $p < 0.05$). Differences in metal concentrations in the organs of depurated bivalves were compared using an ANOVA ($p < 0.05$). When the general variance model was significant, multiple comparisons were conducted using the Tukey HSD test. A Pearson correlation analysis was performed to determine if there was a relationship between Cd concentrations in the gills and bivalve filtration rates. Statistical analyses were performed using SYSTAT and SigmaPlot software (Systat Software Inc., Chicago, Illinois).

Bioaccumulation model

Parameterisation

Kinetic models of metal bioaccumulation are based on a simple conceptual model in which the concentration of a

Table 2. Summary of the values used for the parameters in the biodynamic model for the simulation of cadmium accumulation by *Pygmaodon grandis* under field conditions.

Parameter	Value	Explanation
Apparent α_w gills	0.12	
Apparent α_w digestive gland	0.06	
Whole animal α_w	0.08	
Filtration rate (FR)	0.58 L·d^{-1}·(g bivalve fresh mass)$^{-1}$	
Apparent AE gills	0.04	
Apparent AE digestive gland	0.71	
Whole animal AE	0.57	
Ingestion rate (IR)	6.5–13.9×10^{-4} g dry mass algae·d^{-1}·(g bivalve fresh mass)$^{-1}$	Estimated IR, based on FR = 1.9 [L·d^{-1}·(g bivalve fresh mass)$^{-1}$] and [algae] (g algal dry mass·L^{-1})
$[Cd]_w$	0.3 nmol·L^{-1}, 0.06–0.57 nmol·L^{-1}	Mean value and range of [Cd] for the nine lakes studied by Bonneris et al. (2005).
$[Cd]_f$	3–28.9 nmol·(g dry mass)$^{-1}$	Estimated range of [Cd] in phytoplankton, based on the measured $[Cd]_w$ value; ($[Cd]_f = 76\times[Cd]_w/1.5$), where 76 = [Cd] in *P. subcapitata* and 1.5 = $[Cd]_w$ in equilibrium with the labelled alga.
[algae]	320–720 µg dry mass·L^{-1}	Estimated concentration range for algae and algal detritus, based on pigment concentrations (chlorophyll *a* and phaeo-pigments) measured in the nine lakes studied by Bonneris et al. (2005).
k_e	0.0022·d^{-1}	From Tessier et al. (1987)
k_g	0.00044·d^{-1}	From Perceval et al. (2004)

Note: Square brackets "[]" indicate that the parameter is a concentration; AE, assimilation efficiency (0.01→1.0) from ingested food (unitless); α_w, Cd absorption efficiency (0.01→1.0) from the dissolved phase (unitless).

metal in an organism is controlled by the balance between metal uptake, metal elimination, and organism growth. Thus Cd concentrations in a bivalve can be described by the following equation (Landrum et al. 1992):

$$(1)\quad [Cd]_{bivalve} = \left[\left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + k_g}\right)\cdot\left(1 - e^{-(k_{ew}+k_g)t}\right)\right] + \left[\left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + k_g}\right)\cdot\left(1 - e^{-(k_{ef}+k_g)t}\right)\right]$$

where $[Cd]_{bivalve}$ is the Cd concentration in a bivalve [nmol·(g fresh mass)$^{-1}$], α_w is the Cd absorption efficiency (0.01→1.0) from the dissolved phase (unitless), FR is the filtration rate [L·d^{-1}·(g bivalve fresh mass)$^{-1}$], $[Cd]_w$ is the dissolved Cd concentration (nmol·L^{-1}), AE is the Cd assimilation efficiency (0.01→1.0) from ingested food (unitless), IR is the ingestion rate [g algal dry mass·d^{-1}·(g bivalve fresh mass)$^{-1}$], $[Cd]_f$ is the Cd concentration in ingested particles [nmol·(g dry mass)$^{-1}$), k_{ew} and k_{ef} are the efflux rate constants for Cd taken up from water and food, respectively (d^{-1}), and k_g is the growth rate constant (d^{-1}). In this equation, Cd uptake includes contributions from food (AE · IR · $[Cd]_f$) and water (α_w · FR · $[Cd]_w$).

At steady state, metal uptake is balanced by metal elimination and bivalve growth such that bivalve Cd concentrations are constant, that is,

$$(2)\quad [Cd]^{ss}_{bivalve} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + k_g}\right) + \left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + k_g}\right)$$

where $[Cd]^{ss}_{bivalve}$ equals the steady state Cd concentration in a bivalve.

Filtration rates were determined from the volume of water cleared of algae by a bivalve per unit time according to Coughlan's equation (Coughlan 1969; Tran et al. 2000):

$$(3)\quad FR = \frac{V \cdot \{[\ln(d_o) - \ln(d_f)] - [\ln(d_o') - \ln(d_f')]\}}{(t \cdot M)}$$

where FR is the filtration rate [L·h^{-1}·(g bivalve fresh mass)$^{-1}$]; V is the volume of water in each beaker (2 L); (d_o) and (d_f) are algal densities (cells·mL^{-1}) at the beginning and end of the measurement period, respectively; (d_o') and (d_f') are algal densities (cells·mL^{-1}) in the control chamber without a bivalve, at the beginning and end of the measurement period, respectively; t is the duration of each exposure period (h); and M is body mass (fresh mass, g). Equation 3 assumes that decreases in algal density are due only to bivalve filtration and that all algae passing through the mantle cavity are retained by the bivalve.

Cadmium absorption efficiency from the dissolved phase (α_w) represents the proportion of dissolved cadmium that the bivalve accumulated with respect to the total that it pumped through the mantle cavity over a given time period:

$$(4)\quad \alpha_w = (\Delta[Cd]_{bivalve}/FR \cdot t \cdot [Cd]_w\ )$$

where α_w is the Cd absorption efficiency from the dissolved phase (unitless), $\Delta[Cd]_{bivalve}$ is the increase in Cd concentration in the bivalve following Cd-exposure [nmol·(g fresh mass)$^{-1}$], FR is the filtration rate [L·h^{-1}·(g fresh mass)$^{-1}$], and t is the exposure duration (60 h), and $[Cd]_w$ is the dissolved Cd concentration (nmol·L^{-1}). Absorption efficiencies (apparent α_w) were also estimated for the gills and digestive gland. The use of the word "apparent" refers to the fact that an internal organ such as the digestive gland does not interact with waterborne Cd directly, yet does accumulate some of the dissolved Cd that passes through the pallial cavity.

Hourly bivalve ingestion rates were calculated for each individual bivalve using the amount of algae consumed by the bivalve during the 4 h feeding period, and taking into account algal growth in the control beakers without bivalves:

$$(5)\quad IR = \left\{ V \times \frac{[(d_0 - d_f) + (d_f' - d_0')]}{t} \times 1.8 \times 10^{-12} \right\} \times (1/M)$$

where IR is the ingestion rate [g algal dry mass·h^{-1}·(g bivalve fresh mass)$^{-1}$]; V is the volume of water in each beaker (2 L); d_0 is the initial algal density at the beginning of the feeding period (cells·mL^{-1}); d_f is the final algal density at the end of the feeding period (cells·mL^{-1}); d_0' is algal density (cells·mL^{-1}) in the control beaker without a bivalve, at the beginning of the feeding period; d_f' is algal density (cells·mL^{-1}) in the control beaker without a bivalve, at the end of the feeding period; t is the duration of the exposure period; M is body mass (fresh mass, g) and 1.8×10^{-12} is the mass of one algal cell (g dry mass·cell^{-1}).

Assimilation efficiencies from food were calculated for each bivalve by dividing the total amount of Cd accumulated by the bivalve by the amount of Cd that the bivalve had ingested in the 16 h period (with the assumption that no Cd was lost during this time — cf. Tessier et al. (1987)):

$$(6)\quad AE = \Delta[Cd]_{bivalve}/(IR \cdot [Cd]_f \cdot t)$$

where AE is the assimilation efficiency of Cd per bivalve (unitless); IR is the mean ingestion rate measured for each bivalve over four 4 h feeding periods [g algal dry mass·h^{-1}·(g bivalve fresh mass)$^{-1}$]; $[Cd]_f$ is the mean Cd concentration in the algae [nmol·(g dry mass)$^{-1}$]; t is the length of the feeding experiment ($4 \times 4 = 16$ h); and $\Delta[Cd]_{bivalve}$ is the increase in Cd concentration in the bivalve [nmol Cd·(g bivalve fresh mass)$^{-1}$] that occurred during the feeding experiment. $[Cd]_{bivalve}$ was measured after an 8 d depuration period. As with α_w, the calculated value is multiplied by 100 for presentation as a percentage (%) in figures and tables. We also estimated assimilation efficiencies for the gills and digestive gland, which in these cases represent the proportion of the ingested Cd that crosses the gut wall and then is transported to and accumulated in these organs; these estimates will be referred to as "apparent AE" values for the gills or digestive gland.

Simulations

The model was first used to calculate the relative importance of waterborne and diet-borne Cd as sources of this metal for *P. grandis* held under laboratory conditions. We then explored the effects of varying assimilation efficiency (AE), Cd concentration in the food and algal density on the relative importance of food as a source of Cd for whole *P. grandis*, to determine how sensitive the simulations were to variations in these parameters. Finally, we moved from the laboratory into the field and ran the model with water chemistry data from earlier field campaigns to predict the Cd concentrations in the gills and digestive gland of native specimens of *P. grandis* living in lakes on the Canadian Precambrian Shield. The values that we used for the various model parameters are summarized (Table 2).

For our field applications of the bioaccumulation model, in addition to the model parameters determined in the laboratory (i.e., α_w, AE, and bivalve filtration rate), we also required input values for $[Cd]_w$, $[Cd]_f$, and [algae]. For the initial simulation we used a value of 0.3 nmol·L^{-1} for the ambient dissolved Cd concentration, i.e., the average value reported by Bonneris et al. (2005) for nine lakes in the Rouyn-Noranda area in northwestern Quebec, Canada. The value for $[Cd]_f$ was calculated on the basis of the Cd concentration measured in *P. subcapitata* [76 nmol Cd·(g dry mass)$^{-1}$], adjusted for an ambient Cd concentration of 0.3 nmol·L^{-1} rather than 1.5 nmol·L^{-1} (the residual Cd concentration remaining in solution in the algae labelling experiment), i.e., $(0.3/1.5) \times 76 = 15$ nmol Cd·(g dry mass)$^{-1}$. Finally, to estimate [algae] we used the chlorophyll *a* and phaeopigment concentrations measured during the ice-free season in the lakes Rouyn-Noranda lakes that were studied by Bonneris et al. (2005). Based on these data (P.G.C. Campbell, Y. Couillard, A. Giguère, and O. Perceval, unpublished data, summer 1997), live phytoplankton levels may be between 60 and 190 µg·L^{-1} (dry mass) in the water columns of these lakes. Furthermore, concentrations of phaeopigments are relatively high in these lakes, ranging from 2 to 5 times the chlorophyll concentrations (phaeopigments refers to dead algae, still presumably available to filter-feeding bivalves). It follows that the total concentration of algal cells (living and dead) in the water columns of the Rouyn-Noranda lakes could be as high as 1100 µg dry mass·L^{-1} [i.e., $(5+1) \times 190$].

During the feeding experiments, the bivalves were exposed to ~400 000 algal cells·mL^{-1} or ~730 µg·L^{-1} (dry mass). In other words, algal densities in the feeding experiments were already at the high end of what might be found in the Rouyn-Noranda lakes; increasing the algal densities to 1100 µg·L^{-1} would only increase the ingestion rate by a factor of about 1.5 (1100/730). On the basis of these calculations, we used a maximum ingestion rate of 80 µg algal dry mass·h^{-1}·(g bivalve fresh mass)$^{-1}$ or 5.3×10^{-4} g algal dry mass·d^{-1}·(g bivalve fresh mass)$^{-1}$ [i.e., the maximum ingestion rate observed experimentally (54 µg algal dry mass·h^{-1}·(g bivalve fresh mass)$^{-1}$] multiplied by the calculated factor of 1.5). For this first simulation, which was based on our laboratory experiments, we assumed that Cd efflux rates and bivalve growth rates were negligible.

In the second field application, we applied the bioaccumulation model not to a generic Canadian Shield lake, but rather to each of the lakes that had been sampled by Bonneris et al. (2005). The model was used to calculate the steady-state concentrations of Cd in the gills and in the digestive gland, for bivalves living in each lake, and the predicted concentrations were then compared with the observed Cd concentrations in these organs, as reported by Bonneris et al. (2005). For these field calculations, we used an efflux rate (k_e) of 0.0022·d^{-1}, based on the biological half-life of Cd in *P. grandis* (315 d) as determined by Tessier et al. (1987) ($k_e = 0.693/t_{1/2}$). A growth rate constant (k_g) of 0.00044·d^{-1} was used, as determined by Perceval et al. (2004) for bivalves in the Rouyn-Noranda area.

Results

Accumulation of aqueous cadmium

Concentrations of aqueous Cd declined during each 20 h exposure period, owing to uptake by the bivalves and passive adsorption to their shells. In general, aqueous Cd concentrations decreased to about half of their initial values by the end of the 20 h exposure period. These losses were proportionately greater for the low Cd concentrations (mean 55%) than for the high concentrations (mean 35%).

Cadmium accumulation in whole bivalves increased as the dissolved Cd concentration in the exposure medium increased (Table 3). The highest Cd concentrations were generally observed in the gills, followed by the digestive gland, mantle, miscellaneous organs, and the foot, respectively (Table 3). Approximately 50% of the total Cd burden of *P. grandis* was found in the gills, followed by the mantle (~20%), the miscellaneous organs (~20%), the digestive gland (~10%), and the foot (3%) (Table 4). An ANOVA performed on the arcsine-transformed percentages showed that the proportion of Cd in each organ was insensitive to the dissolved Cd concentrations in the exposure medium. Thus, the relative contributions of the gills and mantle to the total Cd burden did not change significantly as the dissolved Cd concentration increased. For the digestive gland, foot and miscellaneous organs, the proportions were somewhat more variable (Table 4), but this variability was not related to the exposure regime.

Individual filtration rates ranged from 1–140 $mL \cdot h^{-1} \cdot (g\ fresh\ mass)^{-1}$, for a mean filtration rate of 24 ± 17 $mL \cdot h^{-1} \cdot (g\ fresh\ mass)^{-1}$ (± SE) (Table 5). For bivalves exposed to 0.1, 0.5, and 5 $nmol \cdot L^{-1}$ dissolved Cd, cadmium concentrations in *P. grandis* gills were positively correlated with the bivalve filtration rates that were determined during the daily 4 h feeding periods — animals with the higher filtration rates accumulated higher concentrations of Cd in their gills (Fig. 1). No such relationship was observed in bivalves exposed to the highest dissolved Cd concentration (20 $nmol \cdot L^{-1}$).

The absorption efficiency (α_w) varied (*i*) among individual bivalves for a given Cd concentration (i.e., α_w increased as bivalve filtration rates decreased), and (*ii*) as a function of the Cd exposure concentration. The absorption efficiency of Cd was higher in bivalves exposed to 0.1 $nmol \cdot L^{-1}$ dissolved Cd (19 ± 3 and 10 ± 3 apparent α_w for bivalve gills and digestive gland, respectively, and 12 ± 3 for whole organism (± SE; N = 18)) than in bivalves exposed to 5 $nmol \cdot L^{-1}$ dissolved Cd (3 ± 0.5 and 1 ± 0.1 apparent α_w for bivalve gills and digestive gland, respectively, and 1 ± 0.2 for whole organism (± SE; N = 9)). Because aqueous Cd concentrations of 5 $nmol \cdot L^{-1}$ and 20 $nmol \cdot L^{-1}$ are beyond the range of aqueous Cd concentrations observed in the lakes selected for our field simulations, the absorption efficiency for dissolved Cd by *P. grandis* was calculated using bivalves exposed to 0.1 $nmol \cdot L^{-1}$ and 0.5 $nmol \cdot L^{-1}$ dissolved Cd. The average absorption efficiency for whole bivalves, or apparent α_w values for bivalve gills and digestive gland, were 8 ± 2, 12 ± 2, and 6 ± 1 (± SE; N = 27), respectively (Table 5).

Accumulation of dietary cadmium

At the end of the algal labelling step (after 1 d), ~65% of the dissolved Cd in the algal exposure medium was associated with *P. subcapitata* and Cd concentrations in the algae had reached values of ~76 $nmol\ Cd \cdot g^{-1}$ (dry mass). The residual dissolved Cd concentration was 1.5 $nmol \cdot L^{-1}$ (cf. the range of 0.5–20 $nmol \cdot L^{-1}$ used for the waterborne exposures).

For bivalves exposed to diet-borne Cd and then depurated, the highest Cd concentrations were measured in the digestive gland, followed by the intestines and gills (Table 6). Although Cd was present in the intestines, this may have been due to the presence of undepurated Cd-contaminated algae.

In terms of Cd burdens, ~90% of the total Cd burden was found in the miscellaneous organs and intestines at the end of the 4 d feeding period (i.e., day 0 of depuration; Fig. 2). The relative Cd burden associated with these organs decreased steadily during the 8 d depuration period, whereas the total Cd burden in the gills and digestive gland increased (Fig. 2). At the end of the depuration period, ~40% of the total Cd burden was found in the digestive gland, followed by the gills (~30%), the miscellaneous organs (~20%), the mantle (~10%), the intestines (~2%), and the foot (<1%) (Fig. 2; Table 7). An ANOVA performed on the arcsine-transformed percentages showed no significant difference ($p < 0.05$) in the relative contributions of the gills, digestive gland, mantle, and foot between the two replicate feeding experiments.

To facilitate comparisons among bivalves, we excluded those that did not feed for the full 16 h. Bivalve filtration rates (FR) during the feeding experiments were similar to those determined for the waterborne exposures; they ranged from 8–48 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh mass), for an average of 24 ± 2 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh mass ± SE; N = 14) (Table 5). Bivalve ingestion rates (IR) ranged from 9–54 µg dry mass of $algae \cdot h^{-1} \cdot g^{-1}$ bivalve (fresh mass) for an overall mean of 22 ± 3 µg dry mass of $algae \cdot h^{-1} \cdot (g\ bivalve)^{-1}$ (fresh mass ± SE; N = 14) (Table 5). The average assimilation efficiency (AE) of diet-borne Cd for the whole animal, or apparent AE values for bivalve gills and digestive gland, were 57 ± 9%, 4 ± 3%, and 71 ± 6%, respectively (± SE; N = 14) (Table 5). Assimilation efficiencies of diet-borne Cd for whole organisms decreased as bivalve ingestion rates increased (Fig. 3; $R = 0.88$, $p < 0.001$).

Bioaccumulation model

Water versus food as cadmium sources in the laboratory

The mean filtration rate and absorption efficiency measured during the aqueous Cd exposures and the mean ingestion rate and assimilation efficiency measured during the dietary Cd exposures were used in eq. 2 to determine the relative importance of water and food as sources of Cd for *P. grandis* (Table 8). Note that the denominators in eq. 2, i.e., $(k_e + k_g)$, were considered to be identical (and negligible) for our short-term waterborne and diet-borne exposure experiments, meaning that the relative contributions of food and water can be estimated by comparing the numerators, $(\alpha_w \cdot FR \cdot [Cd]_w)$ and $(AE \cdot IR \cdot [Cd]_f)$. According to the results of these initial simulations, water was the main

Table 3. Mean cadmium concentrations [nmol·(g fresh mass)$^{-1}$ ± SE] for *Pyganodon grandis* exposed to dissolved Cd (nmol·L^{-1}) for 60 h.

Dissolved [Cd] (nmol·L^{-1})	Gills	Digestive gland	Mantle	Miscellaneous organs	Foot
0.1 (N = 9)	0.01±0.002a	0.006±0.001a	0.004±0.001a	0.005±0.001a	0.002±0.001a
0.5 (N = 9×2)	0.06±0.006b	0.02±0.002b	0.02±0.003b	0.02±0.003b	0.005±0.001a
5.0 (N = 9×2)	0.51±0.06c	0.29±0.09c	0.22±0.02c	0.19±0.03c	0.09±0.04b
20 (N = 9)	0.69±0.08d	0.31±0.09c	0.27±0.05c	0.20±0.02c	0.09±0.01b

Note: Different letters beside values denote significant differences among treatments ([Cd]) as determined by analysis of variance (ANOVA) ($p < 0.05$) and compared using the Tukey HSD test.

Table 4. Relative cadmium burdens (% ± SE) observed in *Pyganodon grandis* organs exposed to dissolved Cd (nmol·L^{-1}) for 60 h.

[Cd] (nmol·L^{-1})	Gills	Digestive gland	Mantle	Miscellaneous organs	Foot
0.1 (N = 9)	52±3a	9±1a	17±1a	20±2a	1±0.1a
0.5a (N = 9)	47±3ab	16±1b	17±1ab	14±1b	5±0.9b
0.5b (N = 9)	47±3ab	9±3a	23±1b	18±2ab	3±0.8c
5.0a (N = 9)	48±3ab	8±1a	20±2ab	20±1a	3±0.5c
5.0b (N = 9)	50±2a	10±1a	20±1ab	18±1a	2±0.5a
20 (N = 9)	44±1b	16±2b	20±1ab	15±1b	5±0.5b
Mean (N = 54)	49±2	11±2	19±1	18±1	3±0.5

Note: Different letters beside values denote significant differences among treatments ([Cd]) as determined by analysis of variance (ANOVA) ($p < 0.05$) and compared using the Tukey HSD test.

Table 5. Means (± SE) and ranges in bivalve filtration rates (FR), absorption efficiencies of dissolved cadmium (α_w), ingestion rates (IR), and assimilation efficiencies (AE) of ingested Cd measured during separate waterborne and dietary Cd exposures.

	Mean (± SE)	Range
Dissolved exposure		
Filtration rate (N = 54)		
FR [mL·h^{-1}·(g fresh mass)$^{-1}$]	24±17	<1 to 140
FR [L·d^{-1}·(g fresh mass)$^{-1}$]	0.58±0.41	0.02 to 3.4
Absorption efficiency (N = 27)[a]		
Apparent α_w (%) (gills)	12±2	2 to 40
Apparent α_w (%) (digestive gland)	6±1	1 to 25
α_w (%) (whole body)	8±2	1 to 35
Dietary exposure (N = *14*)		
FR [mL·h^{-1}·(g fresh mass)]	24±2	8 to 48
FR [L·d^{-1}·g^{-1} fresh mass)$^{-1}$]	0.58±0.05	0.19 to 1.1
IR [µg dry mass of algae·h^{-1}·(g fresh mass)$^{-1}$]	22±3	9 to 54
IR [g algal dry mass·d^{-1}·(g fresh mass)$^{-1}$]	5.3×10^{-4}±7.2×10^{-5}	2.2×10^{-4} to 1.3×10^{-3}
Apparent AE (%) (gills)	4±3	1 to 18
Apparent AE (%) (digestive gland)[b]	71±6	21 to >100
AE (%) (whole body)	57±9	5 to >100

Note: N is the number of bivalves; [Cd] = 0.1–5 nmol·L^{-1}. Although FR and IR were measured hourly during the laboratory experiments, to facilitate their use in the bioaccumulation model, these parameters are also expressed in days [i.e., FR: from mL·h^{-1}·g^{-1} (fresh mass) to L·d^{-1}·g^{-1} (fresh mass); IR: from µg algal dry mass·h^{-1}·(g bivalve fresh mass)$^{-1}$ to g algal dry mass·d^{-1}·(g bivalve fresh mass)$^{-1}$].

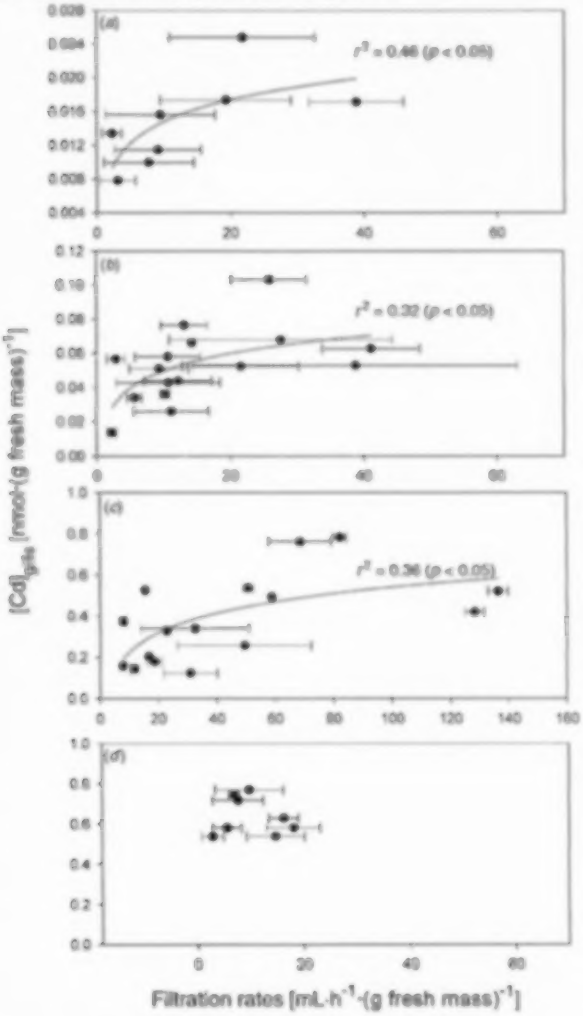
[a]Average values of α_w and of the apparent α_w values for the organs were calculated using bivalves exposed to 0.1 and 0.5 nmol·L^{-1} dissolved Cd (N = 27). The α_w value for the whole organism was calculated on the basis of the sum of the wet mass of all its organs.

[b]In calculating the average apparent AE for the digestive gland and the average AE for the whole animal, the two values that were greater than 100% (see Fig. 3) were set at 100%.

source of Cd for *P. grandis* gills, digestive gland, and the whole organism; food was not an important source of Cd for the gills, but did contribute to Cd accumulation in the digestive gland and the whole bivalve (Table 8).

Given a scenario where AE varies up to 100%, water remains the primary Cd source (compare Table 8, line (*c*) with Table 9, section (*a*)). For food to become the major Cd source, $[Cd]_f$ would have to be 300 nmol·g^{-1} (Table 9, section (*b*)). However, this presupposes that aqueous [Cd] does not increase, which is likely unrealistic. If algal densities were increased from 400 000 cells·mL^{-1} to 2 000 000 cells·mL^{-1} (an upper range for algal densities in lakes of northwestern Quebec; P.G.C. Campbell, Y. Couillard, A. Giguère, and O. Perceval, unpublished data), then ingestion rates would likely increase as would the relative importance of food as a source of Cd (Table 9, section (*c*)).

Fig. 1. Relationship between $[Cd]_{gills}$ and bivalve filtration rates for *Pyganodon grandis* exposed to dissolved Cd: (*a*) 0.1 nmol·L^{-1}; (*b*) 0.5 nmol·L^{-1}; (*c*) 5 nmol·L^{-1}; and (*d*) 20 nmol·L^{-1}. Each dot represents the mean ventilation rate (± SE) for one bivalve. The curves were obtained by non-linear regression.



Prediction of cadmium concentrations in field bivalves

We used the biodynamic model to predict Cd concentrations in the gills and the digestive gland of *P. grandis* in nine northern Quebec lakes, and then compared these predictions with the corresponding values reported in Bonneris et al. (2005). The biodynamic model was reasonably successful in predicting the Cd concentrations in bivalve gills (Table 10); predicted values were similar to those reported for native bivalves by Bonneris et al. (2005), except for Ca-rich lakes where Cd concentrations in the gills were overestimated (e.g., lakes Caron and Ollier). In contrast, predicted Cd concentrations for bivalve digestive glands were generally higher than the Cd concentrations in the digestive gland of *P. grandis* reported by Bonneris et al. (2005), notably for bivalves in moderately contaminated lakes. Possible reasons for this overestimation of steady-state Cd concentrations are discussed below.

Discussion

Aqueous exposure to cadmium

With the exception of the 20 nmol·L^{-1} exposure, the exposure concentrations used for this study fall within the range of those observed in lakes from the Rouyn-Noranda region (Croteau et al. 2002). Our results suggest that the gills are the main target organ when *P. grandis* is exposed to dissolved Cd. Regardless of the exposure concentration, Cd concentrations in the gills were consistently higher than those obtained for the other organs. Despite their relatively modest contribution to the total mass of the organism (23%), the gills accounted for about 50% of the total Cd burden in *P. grandis* after a 60 h exposure. This contribution of the gills to the total Cd burden in *P. grandis* is slightly higher than the average of 40% that was observed by Tessier et al. (1993) in native bivalves collected from contaminated lakes, but falls within the values reported by Bonneris et al. (2005). The mantle, digestive gland and miscellaneous organs contributed 21%, 11%, and 28% to the total Cd burden, respectively, in the bivalves collected by Tessier et al. (1993). Our results are also somewhat higher than those for other bivalve species, as reported by Inza et al. (1997) and Tran et al. (2001) who noted that the gills represented 30% of the total Cd burden for *Corbicula fluminea* (15 d exposure to dissolved Cd), as well as those observed by Roesijadi and Klerks (1989) who reported that the gills represented 37% of the total Cd burden for the oyster *Crassostrea virginica* (14 d exposure to dissolved Cd).

For a given Cd exposure concentration, our results suggest that bivalve ventilation activity influences Cd accumulation from solution. A weak but positive relationship between $[Cd]_{gills}$ and bivalve filtration rates was observed when bivalves were exposed to dissolved Cd concentrations less than or equal to 5 nmol·L^{-1}, i.e., $[Cd]_{gills}$ increased as bivalve filtration rate increased. Note that the bivalve filtration rates plotted in Fig. 1 were not measured during the exposure to waterborne Cd, but rather during the 4 h feeding period, i.e., in the absence of dissolved Cd. For bivalves exposed to the highest dissolved Cd concentration (20 nmol·L^{-1}), there was no relationship between $[Cd]_{gills}$ and filtration rates, but this result may simply reflect the fact that the range of filtration rates was much narrower for these bivalves.

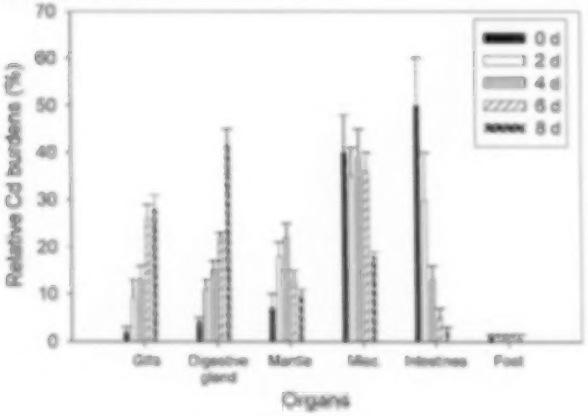
This relationship between $[Cd]_{gills}$ and bivalve filtration rates deserves further consideration. According to the principles of the Biotic Ligand Model (BLM), an increase in gill ventilation should not increase the accumulation of dissolved metals by bivalves (or fish). Within the BLM construct, metal uptake involves three steps: diffusion of the metal from the bulk solution to the biological interface (gill surface), reaction of the metal with a transport site "X" at the gill surface, and movement of the metal across the gill membrane, via the transport site (Gorsuch et al. 2002). The transport of the metal across the apical gill membrane and into the gill cell is postulated to be the slow step in metal uptake. Under such conditions, the gill surface is in equilibrium with the external medium and, therefore, the filtration

Table 6. Mean cadmium concentrations [pmol·(g fresh mass)$^{-1}$ ± SE] in *Pyganodon grandis* exposed to diet-borne Cd and allowed to depurate for 8 d.

Test	Gills	Digestive gland	Mantle	Miscellaneous organs	Intestines	Foot
A (N = 8)	1.38±0.27	10.4±2.2	0.67±0.18	0.97±0.16	1.32±0.20	0.17±0.04
B (N = 9)	2.46±0.44	11.3±1.3	0.89±0.16	0.92±0.13	5.04±1.74	0.25±0.04
Mean	1.95±0.29	10.9±1.2	0.79±0.12	0.94±0.10	3.06±0.88	0.21±0.03

Note: N is the number of bivalves.

Fig. 2. Relative cadmium burdens (% ± SE) observed in *Pyganodon grandis* organs during the 8 d depuration period after exposure of the bivalves to diet-borne Cd.



rate should not influence how much metal is bound to the transport site X or how quickly the metal is accumulated in the gill.

Although our results do not agree with the BLM, they are not unprecedented — other studies have shown that gill ventilation may influence metal uptake in aquatic animals. For example, Tran et al. (2000, 2001) noted an increase in Cd uptake in the freshwater bivalve *Corbicula fluminea* when the animals were exposed to hypoxic conditions. A reduction in dissolved oxygen concentrations caused an increase in filtration rates and a concomitant increase in Cd accumulation in the gills. Hypoxia also induced a change in the Cd burden in the gills. During a 15 d exposure to dissolved Cd (2 μg·L^{-1}; 18 nmol·L^{-1}), Tran et al. (2001) observed that the proportion of the total Cd burden in the gills increased from 13.1 ± 2.8% (SE) under normoxia to 31.4 ± 3.7% (SE) under hypoxic conditions. For hydrophobic micro-pollutants (where diffusion across the gill membrane is rapid and transport to the gill surface may limit uptake rates), it has been hypothesized that an increase in gill filtration rates leads to a higher water flow over the gills and a thinner unstirred boundary layer, thus explaining the increase in the accumulation of the contaminant in the gill (Sijm et al. 1995). However, this hypothesis is clearly incompatible with the BLM, where metal transport across the gill membrane is the slow step.

In the present case, we suggest that the effects of bivalve filtration rates on Cd uptake might not reflect a hydrodynamic phenomenon (thinner unstirred layer at the gill surface) but rather an increase in the gill surface area that is irrigated by increased blood flow within the gills or that is exposed to increased water flow over the gill surface. If the bivalves are exploiting a larger surface area of the gills, then we could expect a higher rate of metal uptake. Also, a decrease in overall filtration rates, as measured over a 4 h period, could be a consequence of the bivalve's behaviour (e.g., the bivalve could be closed for lengthier periods of time). In this case, Cd concentrations in the pallial cavity would decrease while the bivalve is closed, reducing the Cd-exposure concentration and Cd uptake in the bivalve.

Dietary cadmium uptake

The digestive gland was the main target organ when *P. grandis* was exposed to dietary Cd. Harvey and Luoma (1985) also reported that Cd concentrations were highest in the digestive gland of *Macoma balthica* after a 22 d exposure to radiolabelled bacteria. In addition, Graney et al. (1984) and Wang et al. (1996) noted that following dietary Cd exposure, concentrations of freshly accumulated Cd were higher in the digestive gland than in the other organs of *C. fluminea* and *M. edulis*.

Even when we exposed bivalves to Cd in the diet alone, after 8 d 30% of their newly accumulated Cd was located in the gills, presumably reflecting transport from the digestive gland via the circulatory system. Tran et al. (2002) also noted that, after feeding *C. fluminea* a Cd-contaminated diet, 60%–70% of the total Cd burden was located in the visceral mass (which contains the digestive gland), followed by the gills (15%–20%), the muscles and foot (9%–15%), and the mantle (6%–8%). In addition, Wang et al. (1996) noted that after feeding *M. edulis* radiolabelled algae for 7 d, >60% of the total Cd burden was located in the digestive gland, followed by the mantle (~30%), the foot and muscles (~10%), and the gills (<5%).

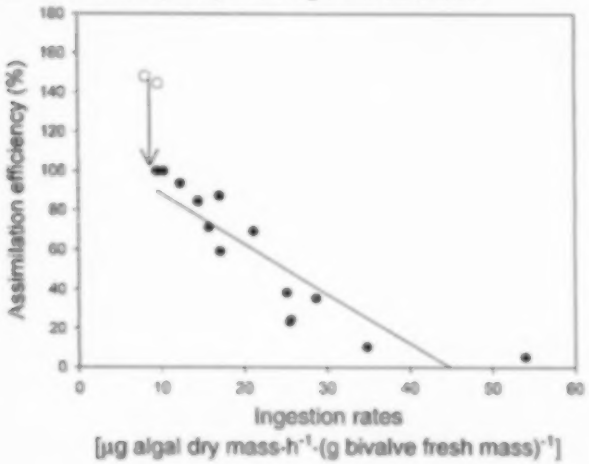
Ingestion rates in our study are lower than those reported for many marine bivalves, which can be over 1000 μg dry mass·h^{-1}·(g fresh mass)$^{-1}$ (Wang et al. 1995; Fisher et al. 1996; Wang et al. 1996), but are higher than the 3–12 μg dry mass·h^{-1}·(g fresh mass)$^{-1}$ range reported for *Corbicula fluminea*, a freshwater mussel (Tran et al. 2002). Although ingestion rates had little effect on the total amount of Cd accumulated in the digestive gland of *P. grandis*, unlike the relationship observed between dissolved Cd and $[Cd]_{gills}$, ingestion rates did significantly influence the assimilation efficiency of dietary Cd in *P. grandis*. In general, AE was higher when ingestion rates were low, suggesting that lower ingestion rates allow more complete digestion of the algal food and higher assimilation of the associated Cd. Similar relationships between AE and ingestion rates have been reported for other mussel species (Wang and Fisher 1996; Chong and Wang 2000*b*), as well as gastropods (Cheung

Table 7. Relative cadmium burdens (% ± SE) observed in *Pyganodon grandis* organs after exposure of the bivalves to diet-borne Cd and depuration for 8 d.

Test	Gills	Digestive gland	Mantle	Miscellaneous organs	Intestines	Foot
A (N = 8)	24±4a	46±6a	12±2a	15±2a	3±0.5a	0.6±0.1a
B (N = 9)	29±2a	38±2a	11±1a	20±1b	0.6±0.1b	0.7±0.1a
Mean	27±2	42±3	11±1	18±1	2±0.4	0.6±0.1

Note: N is the number of bivalves. Different letters beside values denote significant differences between the two replicate experiments, A and B, as determined by one-way analysis of variance (ANOVA) ($p < 0.05$) and compared using the Tukey HSD test.

Fig. 3. Relationship between cadmium assimilation efficiency (AE) in the whole animal (%) and bivalve ingestion rates [µg dry mass of algae·h^{-1}·(g bivalve fresh mass)$^{-1}$] for *Pyganodon grandis* exposed to dietary Cd. Note: The two AE values that exceeded 100% were set at 100% (arrow) for the regression calculation.



and Wang 2005) and zooplankton (Munger and Hare 2000; Ponton and Hare 2009).

Bioaccumulation model

Limitations of the modeling exercise

Uptake of Cd by *P. grandis* from solution is dependent on the dissolved Cd concentration, the metal's absorption efficiency and the bivalve's filtration rate. Filtration rates for *P. grandis* were measured using only one algal species and at a reasonably constant initial algal concentration. Many factors, including food quality and quantity, have been shown to influence bivalve filtration rates. For example, lower algal densities have been shown to lead to increased bivalve filtration rates, as demonstrated by Tran et al. (2002) and Fournier et al. (2005) for *C. fluminea*. Changes in filtration rates might be expected to have relatively little effect on the relative importance of water and food as sources of Cd for *P. grandis*, since an increase in filtration rate would increase both the amount of dissolved Cd and the number of algal cells that enter the mantle cavity. However, this reasoning assumes that α_w is constant (8% for whole organism), regardless of the filtration rate, and thus it may overestimate the importance of waterborne Cd at high filtration rates; under real-world conditions, α_w would likely decrease at high filtration rates, a trend that has been reported for *C. fluminea* (Tran et al. 2001).

The absorption efficiency of aqueous Cd by whole *P. grandis* (average, 8%; range, 1%–35%) is higher than the values of α_w reported for Cd for marine species, which range from 0.2% to 5% depending on the bivalve species (Wang et al. 1996; Chong and Wang 2001; Wang 2001). Our results do, however, encompass the range of those observed for the freshwater bivalve *C. fluminea* by Tran et al. (2002), who reported absorption efficiencies that varied from 2% to 17% depending on filtration rates, water temperature, and food density. It is perhaps not surprising that the absorption efficiency of dissolved Cd is higher in freshwater species than their marine counterparts, given that Cd is strongly complexed by chloride in seawater, reducing its bioavailability and hence its uptake in marine invertebrates (Langston and Bebianno 1990).

One potential limitation of our study is that the bivalves were fed a pure suspension of one algal species during the food exposures. Previous studies have demonstrated that the assimilation efficiency of metals may be influenced by food type, algal assemblages, and whether sediments are present in the suspended particulate matter (Lee and Luoma 1998). The AE of metals associated with sediments is generally much lower than the AE from algae and diatoms (Gagnon and Fisher 1997; Griscom et al. 2000; Chong and Wang 2000*a*). Lee and Luoma (1998) demonstrated that the assimilation efficiencies of Cd were higher in *P. amurensis* and *M. balthica* when the mussels were fed contaminated algae (26%–45% for *P. amurensis*; 33%–51% *for M. balthica*) than when they were fed a sediment–algae mixture (8%–16% for *P. amurensis*; 8%–20% *for M. balthica*). This variability in assimilation efficiencies as a result of changes in food quality and algal assemblages has also been noted in freshwater systems, notably for the mayfly *Centroptilum triangulifer*; Xie et al. (2010) noted that seasonal differences in periphyton flora strongly influenced Cd uptake in *C. triangulifer*. *Pyganodon grandis* has been observed ingesting non-living material in the laboratory (e.g., latex beads) and sediments have been found in the gut of bivalves collected from the field (Tessier et al. 1984), suggesting that these animals do ingest suspended matter rather indiscriminately. The assimilation efficiency for particulate Cd in *P. grandis* may thus be different in the field than in our laboratory experiments, owing to the presence of sediments and various algal species in the suspended particulate matter.

The relative importance of water and food as sources of Cd to *P. grandis* will depend not only on the values of α_w and AE, as discussed above, but also on the concentrations of waterborne and diet-borne Cd to which the bivalve is exposed ($[Cd]_w$ and $[Cd]_f$, respectively). Direct comparisons of these variables are not possible because their units are dif-

Table 8. Relative importance (%) of water and food as sources of cadmium (gills, digestive gland, and whole organism) for the freshwater bivalve *Pyganodon grandis*.

	Uptake from water[a]			Uptake from food[b]			Relative importance[c]	
	$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food
(*a*) Gills	0.3	0.58	12	15	5.3×10^{-4}	4	98	2
(*b*) Digestive gland	0.3	0.58	6	15	5.3×10^{-4}	71	64	36
(*c*) Whole bivalve	0.3	0.58	8	15	5.3×10^{-4}	57	75	25

[a]$[Cd]_w$, dissolved Cd concentration (0.3 nmol·L^{-1}; average value reported by Bonneris et al. (2005) for nine lakes in the Rouyn-Noranda area in northwestern Quebec, Canada); FR, mean filtration rate [L·d^{-1}·(g fresh mass)$^{-1}$]; α_w, mean absorption efficiency of dissolved Cd (%).

[b]$[Cd]_f$, mean Cd concentration in algae [nmol·(g dry mass)$^{-1}$] calculated according to the relation $[Cd]_f = 76 \times ([Cd]_w/1.5)$, where 76 = the Cd concentration in *P. subcapitata* [nmol Cd·(g dry mass)$^{-1}$] attained in the labeling experiment, and 1.5 = the Cd concentration in solution (nmol·L^{-1}) at the end of the labeling step; IR, mean ingestion rate [g algal dry mass·d^{-1}·(g bivalve fresh mass)$^{-1}$]; AE, mean assimilation efficiency of Cd from food (%).

[c]Uptake from water = $\alpha_w \cdot FR \cdot [Cd]_w$, and uptake from food = $AE \cdot IR \cdot [Cd]_f$; see eq. 2.

Table 9. Relative importance (%) of water and food as sources of cadmium (whole organism) for the freshwater bivalve, *Pyganodon grandis* — influence of assimilation efficiency (AE), Cd concentration in food ($[Cd]_f$), and ingestion rate (IR; 5× algal density).

	Water			Food			Relative importance	
Changes	$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food
(*a*)								
Higher AE	0.3	0.58	8	15	5.3×10^{-4}	100	64	36
Lower AE	0.3	0.58	8	15	5.3×10^{-4}	1	99	1
(*b*)								
Higher $[Cd]_f$	0.3	0.58	8	300	5.3×10^{-4}	57	13	87
Lower $[Cd]_f$	0.3	0.58	8	1	5.3×10^{-4}	57	98	2
(*c*)								
Higher IR (5× algal density)	0.3	0.58	8	15	2.6×10^{-3}	57	40	60

Note: $[Cd]_w$, dissolved Cd concentration (nmol·L^{-1}); FR, mean filtration rate [L·d^{-1}·(g fresh mass)$^{-1}$]; α_w, mean absorption efficiency of dissolved Cd (%); $[Cd]_f$, Cd concentration in algae [nmol·(g dry mass)$^{-1}$]; IR, mean ingestion rate [g algal dry mass·d^{-1}·(g fresh mass)$^{-1}$]. Note that in section (*c*) the IR was increased 5-fold, reflecting an increase not in ventilation rate, but in algal density; AE, assimilation efficiency of Cd from food (%) (uptake from water = $\alpha_w \cdot FR \cdot [Cd]_w$, and uptake from food = $AE \cdot IR \cdot [Cd]_f$; see eq. 2).

ferent. However, we can estimate the concentrations of Cd in each medium in a given volume of water in the laboratory experiments as follows. The Cd concentrations in the food [76 nmol Cd·(g dry mass)$^{-1}$] and the concentrations of food (~1 mg algal fresh mass·L^{-1}) can be converted into concentrations of particulate Cd in the dietary exposure flasks (~70 pmol Cd·L^{-1}). Comparing this value with those for dissolved Cd (100–500 pmol Cd·L^{-1}), it is obvious that the bivalves were exposed to higher concentrations (up to 7× more) of dissolved Cd than to particulate Cd in our experiments.

To determine whether this large difference between the Cd concentrations of dissolved and particulate Cd is environmentally realistic, we considered the average Cd concentrations in the algae used during the feeding experiments. Using the K_D for Cd as calculated by Sigg (1987) for suspended particles collected in two Swiss lakes [50–500 L·(g dry mass)$^{-1}$], and considering dissolved Cd concentrations ranging from 0.1 nmol·L^{-1} to 0.5 nmol·L^{-1}, one can calculate that the concentration of Cd in the particulate phase would range from 5 nmol Cd·g^{-1} to 250 nmol Cd·(g particles)$^{-1}$ (dry mass) [e.g., (0.1 nmol·L^{-1})·(50 L·g^{-1}) = 5 nmol·g^{-1}]. The Cd concentration in the algae used in the present study was ~76 nmol·(g dry mass)$^{-1}$. This value falls within the calculated range and suggests that the algae were not unreasonably contaminated during our experiments and that the algal Cd concentrations approach those observed in a natural setting.

Model application in the field

For our initial application of the bioaccumulation model, we ran a simulation for a whole bivalve from a generic Canadian Shield lake. Under these simulated field conditions, dissolved Cd was the main source of Cd for *P. grandis*. This observation is consistent with the results reported by Tessier et al. (1993) who observed a positive relationship between $[Cd]_{bivalve}$ and concentrations of the free Cd ion, $[Cd^{2+}]$, in 38 Quebec lakes. However, such a correlation does not rule out food as a possible source of Cd to *P. grandis*, since Cd concentrations in the food (e.g., phytoplankton) would presumably be correlated with those of dissolved Cd. Our results also agree with those of Wang et al. (1996), who reported that Cd was predominantly accumulated from the dissolved phase by the blue mussel, *Mytilus edulis*. Our results do contrast, however, with those reported by Roditi et al. (2000) who suggested that the zebra mussel, *Dreissena polymorpha*, accumulated Cd mainly from ingested particles.

In the second field application, we applied the bioaccumulation model not to a generic Canadian Shield lake, but rather to each of the lakes that had been studied by Bonneris et al. (2005). Input values for $[Cd]_w$, $[Cd]_f$, and algal den-

Table 10. Comparison between calculated steady-state cadmium concentrations in the gills and digestive gland of *Pyganodon grandis* and those measured in native bivalves collected from lakes in the Rouyn-Noranda region (Bonneris et al. 2005).

	Water[a]		Food[b]				Gills			Digestive gland		
Lake	$[Cd]_w$	$[Ca]_w$	[chl-*a*]	$[Cd]_f$	[algae]	IR	Calculated[c] $[Cd]^{ss}$	Measured[d] $[Cd]^{ss}$		Calculated[c] $[Cd]^{ss}$	Measured[d] $[Cd]^{ss}$	
Évain	0.06	166	2.88	3.0	719	1.4×10^{-3}	132	160		110	150	
Opasatica	0.07	203	1.63	3.5	408	7.8×10^{-4}	154	95		114	123	
Ollier	0.07	314	1.79	3.5	447	8.6×10^{-4}	154	53		115	55	
Dufay	0.19	71	2.71	9.6	677	1.3×10^{-3}	418	426		343	300	
Jourdès	0.30	174	2.51	15.2	626	1.2×10^{-3}	660	1000		532	325	
Héva	0.34	50	2.22	17.2	555	1.1×10^{-3}	747	641		587	388	
Caron	0.52	275	1.28	26.3	319	6.1×10^{-4}	1139	293		817	245	
Vaudray	0.57	104	2.73	27.9	683	1.3×10^{-3}	1211	1470		995	452	
Bousquet	0.55	76	1.59	28.9	398	7.6×10^{-4}	1250	979		925	969	

[a]$[Cd]_w$, total dissolved Cd concentrations measured in each lake (nmol·L^{-1}), from Bonneris et al. (2005); $[Ca]_w$, total dissolved Ca concentrations measured in each lake (μmol·L^{-1}), from Bonneris et al. (2005).

[b][chl-*a*], chlorophyll *a* concentration (μg·L^{-1}), from Yves Couillard (Environment Canada, 200 Sacre Coeur Boulevard, Gatineau, Québec K1A 0H3, personal communication 2006); $[Cd]_f$, Cd concentration in algae [nmol·(g dry mass)$^{-1}$], calculated for each lake according to the relation $[Cd]_f = 76 \times ([Cd]_w/1.5)$, where 76 = the Cd concentration in *P. subcapitata* [nmol Cd·(g dry mass)$^{-1}$] attained in the labeling experiment, and 1.5 = the Cd concentration in solution (nmol·L^{-1}) at the end of the labeling step; [algae], concentration of algae + algal debris [μg·(L dry mass)$^{-1}$] = ([chl-*a*]/0.02)·5, where 1/0.02 converts chl-*a* to algal dry mass and 5 accounts for the chlorophyll *a*: phaeopigments ratio; IR, ingestion rate = [algae]·FR [g algal dry mass·d^{-1}·(g bivalve fresh mass)$^{-1}$].

[c]Steady state concentration [nmol·(g dry mass)$^{-1}$] calculated according to eq. 2, with FR, mean filtration rate observed in the laboratory = 0.58 L·d^{-1}·(g fresh mass)$^{-1}$; α_w, mean apparent absorption efficiency of dissolved Cd observed in the laboratory = 12% for gills and 6% for digestive gland; AE, mean apparent assimilation efficiency of Cd from food = 4% for gills and 71% for digestive gland; dry mass: fresh mass ratio = 0.05.

[d]Measured whole body concentration from Bonneris et al. (2005) [nmol·(g dry mass)$^{-1}$].

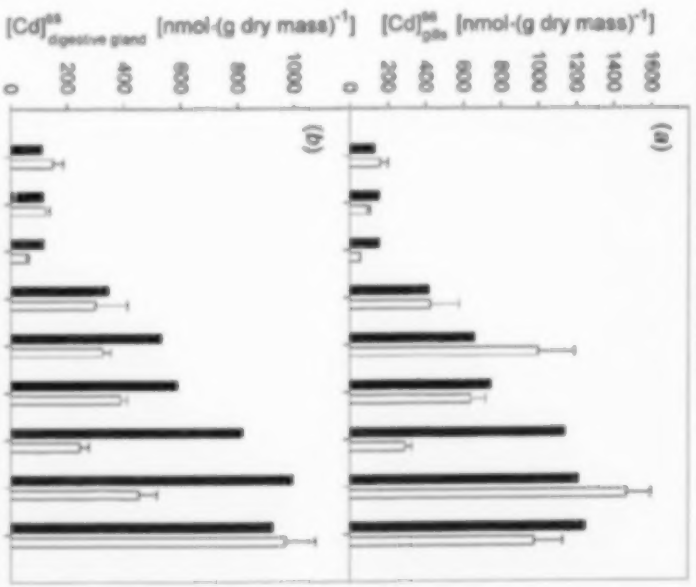


Fig. 4. Comparison between calculated steady-state (*a*) gill Cd concentrations (± SD) and (*b*) digestive gland Cd concentrations (± SD) in *P. grandis* exposed to waterborne + diet-borne Cd (filled bars) and those measured (open bars) in the gills and digestive glands of bivalves collected from lakes in the Rouyn-Noranda region (Bonneris et al. 2005). See Tables 2 and 10 for details of the simulation. The two-letter lake codes correspond to the names given in Table 10 and are in the same order.

sites are provided, along with a summary of the calculations used to estimate these values (Table 10). Steady-state Cd concentrations in the bivalve gills, as calculated with eq. 2, were similar to the Cd concentrations measured in the gills of adult bivalves collected from the lakes (Fig. 4*a*). The model did, however, tend to overestimate the Cd concentrations in bivalve digestive glands, notably in the moderately contaminated lakes (Fig. 4*b*). The model results indicate that water is the main source of Cd both for the gills (relative importance water:food::99:1) and for the digestive gland (water:food::80:20).

We note that our laboratory exposures to waterborne and diet-borne Cd did not mimic field conditions exactly. For example, dissolved Ca concentrations varied considerably among our various lakes (50–275 μmol·L^{-1}), but our laboratory exposures were run at a constant Ca concentration (625 μmol·L^{-1}). Higher dissolved Ca concentrations are correlated with lower Cd accumulation in *P. grandis* in the field (Perceval et al. 2002), but our biodynamic model does not currently consider possible protective effects from Ca. Our model also does not take into consideration food quality and composition, as well as bivalve feeding behaviour, fac-

tors that may well influence assimilation efficiency (AE) in the whole organism. It is possible that the apparent assimilation efficiency value used in our model for the digestive gland (71%) may have resulted in an overestimation of Cd concentrations in this organ.

Our modelling results contrast with those reported by Kraemer et al. (2008) for wild yellow perch, *Perca flavescens*, living in the same suite of lakes studied by Bonneris et al. (2005). Their biodynamic model greatly over-predicted steady-state Cd concentrations in wild yellow perch. Based on their results, Kraemer et al. (2008) suggested that *P. flavescens* are able to control Cd accumulation in the gills by invoking a feedback mechanism whereby the perch reduce Cd uptake from the dissolved phase when internal Cd concentrations reach a certain threshold. Our results suggest that *P. grandis* does not have this capacity to control Cd uptake and instead continues to accumulate Cd, albeit slowly.

In conclusion, our results suggest that water is the major source of Cd for the freshwater bivalve *P. grandis*, which implies that changes in its Cd concentrations will be correlated with those of bioavailable waterborne Cd. The corollary is that differences among lakes and over time in the quality and quantity of the suspended material on which this bivalve feeds can be ignored when this animal is used as a Cd biomonitor in the field. Gill Cd concentrations predicted with a biodynamic model were similar to those measured in native bivalves, whereas predicted values for the digestive gland were within a factor of 2 of measured values. These results support the realism of biodynamic modelling as a means of predicting bivalve metal concentrations in nature.

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PERSPECTIVE / PERSPECTIVE

Individual transferable quotas and the "tragedy of the commons"

John Parslow

Abstract: The allocation of individual transferable quotas (ITQs) as shares of a total allowable catch (TAC) is now widely practised in fisheries management, but is not without controversy. It is often suggested that the possession of ITQs should provide an incentive for fishers to exercise stewardship of the resource. Quota holders acting in their economic self-interest should collectively exercise stewardship, setting TACs and supporting enforcement measures to maximize the present value of future profit streams. But it is in the economic self-interest of an individual fisher possessing ITQ to take additional unreported catch, through discarding, high-grading, or quota-busting. Thus, ITQs in themselves will not prevent a "tragedy of the commons", unless there is sufficient compliance monitoring and enforcement to deter hidden catches. ITQs, with adequate enforcement, have been demonstrated to effectively address the race to fish and result in improved sustainability and profitability. There are questions of equity concerning the flow of benefits from the allocations of quotas and associated profit streams and who pays for the management costs required to sustain them. There are also issues around the ability of ITQ-based management to address other social and environmental objectives.

Résumé : L'allocation de quotas individuels transférables (QIT) comme parts du total autorisé des captures (TAC) est communément utilisée dans la gestion des pêches, mais elle n'est pas sans soulever des controverses. On indique souvent que la possession d'un QIT devrait inciter les pêcheurs à exercer une responsabilité de gérance de la ressource. Les possesseurs de quotas agissant pour leurs intérêt personnel devraient exercer collectivement la gérance, en déterminant les TAC et en appuyant les mesures d'application des règlements afin de maximiser la valeur actuelle des perspectives futures de profit. Mais c'est dans l'intérêt économique personnel du pêcheur individuel qui possède un QIT de faire des récoltes additionnelles non signalées, en faisant des rejets, en écrémant les captures ou en dépassant les quotas. Ainsi, les QIT en eux-mêmes n'empêchent pas l'arrivée d'une « tragédie des richesses communes » à moins qu'il n'y ait suffisamment de surveillance du respect des règlements et de leur application pour décourager les prises cachées. On a montré que les QIT, avec une application adéquate, peuvent gérer de façon efficace la course à la pêche et améliorer la durabilité et la profitabilité. Il y a des problèmes d'équité concernant le flux des bénéfices provenant de l'allocation des quotas et des lignes de profit associées et au sujet de qui doit payer les coûts de gestion nécessaires pour les maintenir. Il y a aussi des questions à propos de la capacité d'une gestion basée sur les QIT à atteindre d'autres objectifs sociaux et environnementaux.

[Traduit par la Rédaction]

Introduction

While there is debate over the exact state and likely future trends in global fisheries (Worm et al. 2006; Hilborn 2007*a*), there is general agreement that many stocks are overfished and that there is substantial and damaging overcapacity in global fleets (Clark 2006; Worm et al. 2009). All agree that active management is required to address these problems and that the challenges facing fisheries managers and scientists are considerable (Caddy and Seijo 2005).

Over the last two decades, the allocation of shares of the annual catch in the form of individual transferable quotas (ITQs) has become an increasingly popular fisheries management tool (Squires et al. 1995; Grafton 1996; Grafton et al. 2006). Some have credited the use of ITQs with most of the advances made in the reduction of overfishing and overcapacity in this period (Costello et al. 2008), while others have expressed reservations (Smith et al. 2009).

Bromley (2009) sharply criticises ITQs and the fishery economics underlying them. While his critique is wide-ranging, a key target of his attack is the assertion or assumption that ITQs improve outcomes because they assign individual fishers long-term rights and entitlements to a share of the fishery yield, thereby providing fishers with an incentive for improved stewardship of the resource.

One can certainly find statements to this effect in the ITQ literature (e.g., Squires et al. 1998). However, this literature is also replete with warnings and analyses of practices by ITQ holders, such as discarding, high-grading, and quota-busting (Copes 1986; Turner 1997; Hatcher 2005). These practices are hardly a model of improved stewardship. As

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Copes and Charles (2004) point out, this apparent contradiction reflects a difference between the incentives arising for ITQ holders as a collective and as individuals.

The argument for ITQs has been presented as part of a broader proposition that outcomes are likely to be improved when the incentives for individuals are aligned with management or community objectives (e.g., Hilborn et al. 2005; Hilborn 2007*b*). It is common practice in economics generally, and in fishery economics in particular, to assume that individuals will act in their own rational self-interest by seeking to maximize profits, or in the long term, the expected, discounted present value of the current and future profit stream. In reality, fishers (and human agents more broadly) respond to a broader set of social pressures and values (e.g., Copes and Charles 2004; Gezelius 2004; King and Sutinen 2010). Social pressures may encourage stewardship and discourage selfish behaviours, so that one might expect economic analyses to give a pessimistic rather than optimistic view of individual behaviours under ITQs.

In general, the ITQ literature does present a pessimistic (or perhaps realistic) view of individual practices in ITQ fisheries. Most reviews and analyses conclude that ITQs must be accompanied by strong monitoring and enforcement measures to succeed (e.g., Squires et al. 1998; Branch et al. 2006*a*; Beddington et al. 2007). Theoretical analyses are generally based on the premise that individuals will only comply with regulations around quotas and discarding if the expected costs of noncompliance (probability of detection times penalty) exceed the expected financial returns. In practice, it is generally recognized that a majority of fishers in any fishery tend to be law abiding, and only a minority bases decisions solely on risk and return (Kuperan and Sutinen 1998).

Many of the theoretical analyses of discarding, high-grading, and quota-busting in ITQ-based fisheries consider only returns in the current year. That is, they implicitly assume that individual quota holders take no account of the impacts of current actions on future quota values or future discounted returns. The problem of choosing current and future catches so as to maximize the present value of the future discounted total profit stream from a fishery, taking into account the effect of current catches on future stock size, is well understood (e.g., Clark and Munro 1975; Clark 2006). Arnason (1990, 1994, 2008) considers the interaction between the maximization of collective present value and the maximization of the present value of the discounted profit stream accruing to individual fishers.

Neither Bromley (2009) nor the authors who responded to his paper (Grafton et al. 2009; Harte et al. 2009) provided a direct theoretical analysis of the effects of quotas on stewardship. Here, I examine the maximization of collective and individual present value using a simple model and analysis. I relate these results to more general results derived earlier by Arnason (1990). I present this simple derivation in the hope that it will help to clarify the difference between collective and individual incentives.

A simple model analysis

I use a discrete time version of the Schaefer (1954) model. Here, S_t represents the stock in year t, H_t the harvest in year t, and E_t the effort in year t, and

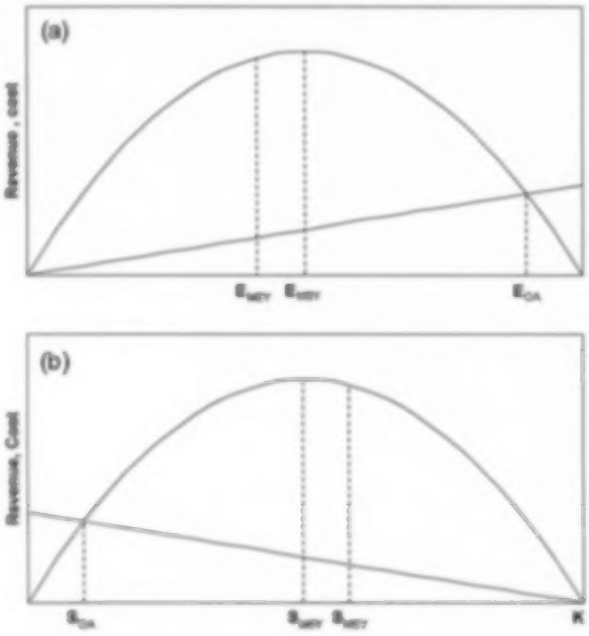

Fig. 1. Total revenue (curved line) and total cost (solid straight line) vs. effort (E; panel *a*) and stock size (S; panel *b*) for the Schaefer (1954) model. S_{OA} and E_{OA} mark the bionomic equilibrium, where excess profit is zero. MEY, maximum economic yield; MSY, maximum sustainable yield; K, maximum stock size.

$$(1) \quad S_{t+1} = S_t + rS_t(1 - S_t/K) - H_t$$

$$(2) \quad H_t = qE_tS_t$$

where r and K are population parameters, assumed fixed, and q is the catchability, also assumed fixed. I assume a fixed unit price p and fixed cost per unit effort c, so the revenue in year t is $R_t = pH_t$, and the total cost $C_t = cE_t$.

At steady state ($S_{t+1} = S_t$):

$$(3) \quad \boldsymbol{H = rS(1 - S/K)}$$

$$(4) \quad \boldsymbol{E = (r/q)(1 - S/K)}$$

$$(5) \quad \boldsymbol{R = pKqE(1 - qE/r)}$$

The Gordon (1954) model is recovered as a steady-state solution. (Bold type is used here to denote values of variables at steady state.) If one plots $\boldsymbol{R}$ vs. $\boldsymbol{E}$ and $\boldsymbol{C}$ vs. $\boldsymbol{E}$ (Fig. 1*a*), the point of intersection represents the combination of $\boldsymbol{E}$, $\boldsymbol{R}$, and $\boldsymbol{C}$ corresponding to the "bionomic equilibrium" $\boldsymbol{E_{OA}}$ in an open access unregulated fishery (Clark 2006). The unit cost c is assumed to include a "normal" level of return on effort and investment, so when $\boldsymbol{R > C}$, there is extra-normal or excess profit available (which might be returned to the resource owner as resource rent). Under open access, one expects additional effort to be invested in the fishery until this excess profit becomes zero.

Plotting $\boldsymbol{R}$ and $\boldsymbol{C}$ vs. $\boldsymbol{S}$ (Fig. 1*b*) offers a complementary picture of the same phenomenon. Again, the open access bi-

onomic equilibrium occurs at a stock size $S_{OA} = c/(pq)$. This has a simple interpretation, since $p - c/(qS)$ is the marginal excess profit at stock size S. Under open access, the stock is fished down until this marginal profit is zero. Note that if price p increases because of demand, and catchability increases or cost per unit effort decreases because of advances in technology, there is nothing to stop the bionomic equilibrium stock size and the corresponding yield being pushed to arbitrarily low levels.

Two other oft-quoted levels of steady-state effort and stock size are indicated in Fig. 1. These are the effort E_{MSY} and stock size S_{MSY} resulting in maximum sustainable harvest or yield and the effort E_{MEY} and stock size S_{MEY} that maximize annual equilibrium profit (revenue – cost).

These are elementary results in fisheries economics. Clark (2006) provides a good introduction and discusses the effects of relaxing some of the simplifying assumptions made above. To take into account future values, one needs to return to the dynamic model. For a (discrete) discount rate d, one can define the present value PV_t of current and future net revenue by

$$(6) \qquad PV_t = \sum_{\tau=0...} (R_{t+\tau} - C_{t+\tau})/(1+d)^{\tau}$$

Consider first the case in which a sole agent (or collective) controls the current and future effort $\{E_{t+\tau}\}$. One would expect the agent to choose a time sequence of effort that maximizes PV_t. For an arbitrary starting point S_t, this is an optimal control problem. Clark (2006) considers this control problem for a continuous time version of the Schaefer eqs. 1 and 2 and shows that the optimal solution is a "bang-bang" solution. There is an optimal steady-state stock size S_{OPT}, and the monopoly should exert the maximum effort available when the stock size exceeds S_{OPT} and zero effort for stock size less than S_{OPT}.

I do not solve the dynamical control problem for the discrete model here, but instead derive an expression for S_{OPT} using a simple variational technique that has the advantage of being easily extended to the case of multiple agents. Suppose the current stock size is S. The sole agent can choose to exert the steady-state effort $E(S)$ this year and indefinitely into the future. This will generate the steady-state $\mathbf{PV}(S)$ given by

$$(7) \qquad \mathbf{PV}(S) = (1 + 1/d)[R(S) - cE(S)]$$

Alternatively, the agent could take an additional harvest δH in this year. This will generate an additional revenue $p\delta H$, at additional cost $c\delta H/(qS)$. The stock next year will be reduced by δH. If the effort next year and in future years is adjusted to maintain the stock at the new level $S - \delta H$, the current value of the steady-state returns next year and in following years is then given by $(1/d)[R(S - \delta H) - cE(S - \delta H)]$. The new **PV**, including the additional catch and cost this year, is

$$(8) \qquad \mathbf{PV}(S, \delta H) = R(S) - cE(S) + [p - c/(qS)]\delta H + (1/d)[R(S - \delta H) - cE(S - \delta H)]$$

If S_{OPT} corresponds to a local maximum of $\mathbf{PV}(S)$, the partial derivative of **PV** (eq. 8) with respect to δH should be zero at S_{OPT}, yielding

$$(9) \qquad p - c/(qS_{OPT}) = (1/d)[R'(S_{OPT}) - cE'(S_{OPT})]$$

This is in fact equivalent to Clark's formula 2.63 derived for the continuous model (Clark 2006, p. 59). Substituting for $R'(S)$ and $E'(S)$ from the Schaefer model gives

$$(10) \qquad d[p - c/(qS_{OPT})] = pr(1 - 2S_{OPT}/K) + cr/(qK)$$

The implications of this result become clearer if one recognizes that the left-hand side of eq. 10 is zero at the bionomic equilibrium S_{OA}, while the right-hand side is zero when equilibrium net profit is a maximum, i.e., at S_{MEY}. One can then rewrite the result as

$$(11) \qquad [d/(2r)](1 - S_{OA}/S_{OPT}) = (S_{MEY} - S_{OPT})/K$$

It follows that the optimum equilibrium monopoly stock size should fall between S_{MEY} and S_{OA}. If the discount rate is much less than the instantaneous population growth rate r (short-lived stocks, low real discount rates), S_{OPT} is close to S_{MEY}. If $d >> r$ (long-lived stocks, high real discount rates), then even a sole agent maximizing present value should choose to push a stock close to S_{OA}. Again, this is all well known and described by Clark (2006) and others.

Now suppose the sole agent is replaced by a set of fishers, all with identical gear and each assigned a fixed share ϕ of the total effort and therefore of the total catch. We now consider the same thought experiment for an individual fisher. The fisher could continue to apply his assigned share ϕ of the effort, receive share ϕ of the catch and therefore the assigned share of the present value, $\phi\mathbf{PV}_{OPT}$. Alternatively, he could choose to exert some additional effort in the current year and thereby receive some additional catch, δH. This will incrementally reduce the stock size and sustainable catch available in the following year. I assume that the total effort is adjusted to the new stock size in subsequent years and that the fisher reverts to taking his assigned share ϕ in future years, so the extra catch is a one-off.

By the same argument as before, his additional net revenue in the current year is $[p - c/(qS)]\delta H$. And as before, the PV of the entire fishery in subsequent years is $(1/d)[R(S - \delta H) - cE(S - \delta H)]$ and decreases by $(1/d)[R'(S) - cE'(S)]\delta H$. But the individual experiences only a fraction ϕ of this decrease. So the individual will increase his present value provided

$$(12) \qquad [p - c/(qS)] > (\phi/d)[R'(S) - cE'(S)]$$

Provided the left-hand side is positive ($S > S_{OA}$), condition 12 will always hold if there are enough shares (that is, ϕ is small enough). That is, in a competitive closed access fishery, with many shares, individual fishers maximizing their individual present value will always take additional catch provided $S > S_{OA}$.

In practice, the future return obtained by the fisher in question will depend not only on his behaviour, but also on the behaviour of all other fishers. The analysis just completed applies in the case where only one fisher chooses to act in his own self-interest and take additional catch. If all fishers made the same decision, and all chose to apply the same additional effort and take the same additional catch, then they would drive the stock to a level below S_{OPT} and would all experience a decrease in PV. This is a classic

problem in game theory, analogous to the prisoner's dilemma (Clark 2006, pp. 140–141; Nash 1951). If an individual increases catch unilaterally, they gain if no one else does and lose if everyone else does the same. If the individual does not increase catch, and anyone else does, the individual loses. The economic theory of competitive equilibrium assumes that competitors faced with such a choice will act selfishly, leading in this case to an open access equilibrium.

To this point, the "rules" controlling the fishery have not been stated explicitly. The fishery might be managed as a closed access fishery, with a limited set of participants, each of whom is free to adjust their effort independently. The preceding analysis would then predict that participants in such a closed access fishery would increase their effort until stock size approaches S_{OA}.

The fishery might be more closely regulated by fixing the effort each participant is allowed to apply. In principle, this fixes E and ϕ and prevents departures from S_{OPT}. In practice, one might expect fishers to increase effective effort by investing to increase catchability q through faster, bigger vessels, improved targeting, and more effective gear. Such unilateral increases in effective effort would also be in the individual's self-interest, even if they came with proportional increases in cost. So one would predict that in a closed access, effort-regulated fishery, there would be a technological race to increase effective effort, which would again push stock size towards S_{OA}, unless the managers were able to detect and compensate for the increase in catchability. This behaviour has long been recognized as a source of failure in input-controlled fisheries management (e.g., Wilen 1979; Branch et al. 2006*b*; Hilborn 2007*b*).

Individual quotas are typically assigned as a share ϕ of an annual catch or TAC, determined by regulators or by the collective monopoly. In principle, an individual can then only take additional catch δH by exceeding the quota, either by taking catch and selling it outside regulated markets (quota-busting) or by high-grading: discarding lower value catch and replacing it by higher value catch. (Discarding may be permitted under fishery regulations, depending on whether quotas are imposed on catches or landings.)

If one assumes that the additional catch is taken in one year only, and that the TAC is then adjusted in the following and future years to match the reduced stock size, then the analysis and conclusions for an individual ITQ holder are identical to those considered previously. An individual taking additional catch will increase their present value provided that condition 12 is met. In practice, if the individual's quota share is small, they should try to take additional catch until stock size approaches S_{OA}. (In a fishery with a small number of quota holders, eq. 12 would predict an equilibrium stock size substantially above S_{OA}.)

This analysis has not explicitly considered the market for ITQ, and in fact the analysis applies to individual quotas, whether or not they are transferable and tradeable. In a situation where all fishers have equivalent costs and gear, the market price of a tradeable quota share should be equal to its present value (Arnason 1990), so the introduction of a quota market should not affect the conclusion. The right-hand side in eq. 12 can be regarded as either the loss of access to future profit if the quota is retained or the reduction in return if the quota is sold.

The above analysis applies to hidden or unreported catch that can still be sold at market price p. It is possible that this catch can only be sold at a discounted price, and then the discounted price should replace the legal price p in the left-hand side of eq. 12. In the case of high-grading, p should be replaced by the price differential between the discarded and retained stock, with allowance made for any differential between handling costs of landed vs. discarded catch (e.g., Arnason 1994). In both situations, the reduction in p will result in an equilibrium stock size S_H that is greater than S_{OA} but lower than S_{OPT}.

The results presented here for a simple model are entirely consistent with those obtained by Arnason (1990) under more general conditions. His conclusions can be summarized as follows:

If we ignore future returns, the current profit for an individual i is maximized when

$$(13) \qquad pH_E(i) = C_E(i)$$

where $H_E(i)$ is the marginal increase in harvest $H[S, E(i), i]$ with increase in effort $E(i)$, and $C_E[E(i), i]$ is the marginal increase in cost $C(i)$ with increase in effort. This condition is met when the net return on an increment of individual catch is zero. Individual fishers obeying this rule will again drive the stock to the bionomic equilibrium.

The present value of the collective profit stream is maximized when

$$(14) \qquad (p - \mu)H_E(i) = C_E(i)$$

Here, μ is the collective shadow price, or effectively the future discounted value of a unit of stock left in the fishery rather than harvested now. Condition 14 is equivalent to eq. 9 in the simple model presented here, and the right-hand side of eq. 9 is just the collective shadow price, i.e., the marginal increase in future value resulting from an increment in stock left unharvested.

The present value of the future profit stream for the individual fisher i is maximized when

$$(15) \qquad [p - \sigma(i)]H_E(i) = C_E(i)$$

The individual shadow price $\sigma(i)$ is less than μ. In fact, if the fishery consists of N identical fishers, it can be shown that $\sigma(i) = \mu/N$. This is equivalent to eq. 12, and the right-hand side of eq. 12 is just the individual shadow price $\sigma(i)$.

Discussion

The preceding analysis shows that the allocation of individual quotas, or equivalent long-term entitlements to a share of future catches, does not provide a sufficient incentive in itself for individual quota holders to refrain from additional hidden effort and harvest. The result is rederived here for a simple model, but has been derived under more general conditions by Arnason (1990). The conclusion is robust, because it depends on the fact, evident in conditions 12, 14, and 15, that an individual fisher captures the immediate benefit of any additional unreported catch, but shares the cost, in terms of reduced future profit, with all other quota holders. Again, this has long been recognized and might be said to be obvious (cf. Copes 1986; Copes and

Charles 2004). It is effectively the "tragedy of the (local) commons".

An interesting conclusion from the analysis is that in the absence of compliance monitoring and enforcement of quotas, the economic incentives involved in choosing catch levels are the same for individual quota holders and for holders of long-term licences in a limited entry fishery. In both cases, individuals have entitlement to a long-term profit stream whose size depends on their collective behaviour, and in both cases, they can increase their present value by increasing individual catches in a way that if practiced by all, drastically lowers collective and individual returns. The key difference of course is that, at least when the quota is imposed on catch rather than landings, fishers can only take additional catch by breaking the rules.

The implication, that adequate compliance monitoring and enforcement is essential for the success of ITQ-based management, is widely recognized in the ITQ literature. A number of papers have assessed the level of monitoring and enforcement required for successful implementation, both on theoretical grounds (Chavez and Salgado 2005; Hatcher 2005; King and Sutinen 2010) and in terms of practical fisheries outcomes (Newell et al. 2005; Branch et al. 2006*a*; Branch and Hilborn 2008). This literature overlaps with the broader literature on illegal, unregulated, and unreported fishing (Gallic and Cox 2006; Sumaila et al. 2006).

It is generally accepted that individual moral stances and social or peer pressures, as well as economic considerations, affect individual decisions about compliance with fishery regulations in general and ITQs in particular. Gezelius (2004) found that social attitudes in small communities depend on the scale and purpose of illegal activities. King and Sutinen (2010) note that the attitudes and decisions of fishers depend on social judgements about the equity and likely success of management actions, potentially creating a vicious cycle in poorly managed fisheries. Transparency and social pressures for compliance may be higher in long-established fishing communities, and this could be weakened or destroyed if quota-trading results in transfer of effort outside local communities and (or) leads to perceptions of inequities.

The successful implementation of ITQs has generally led to increased profitability. Standard economic analyses might suggest that this would increase the reward for noncompliance and therefore require larger investments in monitoring and enforcement or larger penalties (Charles et al. 1999). However, it is also said that noncompliance is more likely in circumstances where profits are low or absent. ITQs create particular perverse incentives for high-grading or discarding in multispecies fisheries or in the presence of price differentials within catches. ITQs for multispecies fisheries are particularly problematic, and a variety of mechanisms have been proposed to address these problems (e.g., Squires et al. 1998). There is evidence that under high levels of enforcement and with adequate trading and adjustment, fishers can target effectively to meet separate TACs on multiple species in mixed-species fisheries (Branch and Hilborn 2008).

If the possession of an ITQ does not in itself provide an incentive for stewardship by individual fishers, and the successful implementation of ITQs requires strong and effective compliance monitoring and enforcement (command and control), then one might ask in what sense ITQs are incentive-based and what advantages they offer over other fisheries management approaches. The weaknesses of other approaches have long been discussed (for recent reviews see Branch et al. 2006*b*; Hilborn 2007*b*). Unregulated open access fisheries rapidly converge on the open access equilibrium S_{OA}. With modern fishing technologies and prices, that will typically involve very low stock levels and very low yields, resulting in low individual and societal benefits, typically exacerbated by overcapitalization along the development trajectory. In limited access fisheries, in the absence of effort controls, effort increases through investment in larger vessels and more effective gear, with the same outcome. Historically, attempts at input effort controls have been defeated by effort creep because of technology improvement. Attempts to impose TACs in competitive fisheries (open access or limited access) typically result in a race to fish and overinvestment in fishing capacity, with fishing seasons often shrinking from months to weeks or days. All of these approaches have resulted in failure to maintain stock at productive levels or dissipation of profit through overcapitalization, or both.

ITQs have arguably been introduced primarily to address the race to fish under competitive TACs and have been successful in doing that, at least where monitoring and enforcement are adequate (Homans and Wilen 2005; Newell et al. 2005; Sanchirico et al. 2006). In the presence of adequate quota enforcement, ITQs offer fishers an incentive to act collectively to maintain quota value in the long term and an incentive to maximize return per unit catch in the short-term by minimizing costs and maximizing market price of the landed product. It is expected that trade in ITQs should lead to increases in overall fleet efficiency, as more efficient fishers buy out less efficient operators. Analyses of successful ITQ schemes have found that much of the increase in profits (and quota values) following the implementation of ITQs has arisen from increases in market price (Homans and Wilen 2005; Newell et al. 2005). By eliminating or reducing the race to fish, ITQs allow catches to be landed in a manner that maximizes value, allowing, for example, the replacement of frozen product landed in a truncated season, by fresh or even live product distributed throughout the year.

In practice, ITQs may not eliminate the race to fish entirely in all fisheries. The simple theoretical analysis presented here ignores within-season stock and quota dynamics. Fishers may enjoy higher effective catch per unit effort (CPUE) by targeting higher stock densities early in the season. Where multispecies fisheries and (or) bycatch are addressed by quota leasing, the price of bycatch quota may increase later in the season.

The possession of quotas provides fishers with an incentive to act collectively to protect and increase quota value, and this can lead to improved co-management between fishers and fishery managers (Hilborn, 2007*b*). Arnason (2008) goes further and argues that under ITQs, industry can and should be allowed to self-manage the fishery, including setting TACs, over-seeing or commissioning the required research and monitoring, and implementing the required compliance monitoring and enforcement. It is in the eco-

nomic, rational self-interest of ITQ holders collectively to do this in a way that maximizes the present value. It is equitable that quota holders should meet the costs of doing this, given that these investments are necessary to maintain the profit stream from the fishery. Arnason (2008) argues that it is also potentially more efficient for industry to do this, as fishers possess much of the information required to manage the fishery better, and this is extracted less efficiently by the institutions (usually government agencies) that currently undertake stock assessment and set TACs.

Most economic analyses assume implicitly that the biological and economic information needed to choose optimal levels of TAC and effort is available to the regulators and fishers. In practice, this information is difficult and expensive to acquire and typically is subject to high levels of uncertainty. Arnason (1990, 2008) considers information requirements and argues that the use of ITQs may reduce information requirements, and (or) encourage cooperative access to information. However, given that much stock assessment still relies implicitly on CPUE, and this may change in unexpected ways because of changes in targeting under TAC and ITQ fisheries, the implications for information reliability and cost are not so clear.

Of course, government agencies have retained the responsibility to set TACs partly because these may be influenced by social and environmental objectives as well as the economic objective of maximizing expected present value. One of the key criticisms levelled at ITQs is that they address economic objectives at the cost of social and environmental objectives (Copes and Charles 2004). These criticisms may be partly addressed by other methods of quota allocation, discussed below.

Arnason (2008) does address the potential problems that might arise in reaching collective agreement among fishers about TACs. As our earlier analysis shows (eq. 9), the optimal stock level depends on the cost structure and discount rate, and these might be expected to vary among individual fishers, leading to different preferred TACs. Arnason argues that these differences should be small enough to allow consensus. He notes that bargaining among individual fishers in setting TACs can be set in a game theory context. One could go further and argue that collective bargaining by fishers over compliance monitoring and enforcement, and decisions by individual fishers as to whether to comply, should also be treated in a game theory context. The key question is whether this game leads to a solution that avoids the "tragedy of the local commons" and leads to a desirable collective outcome.

Some ITQ implementations allow non-fishers to hold quota and lease it annually to fishers. It helps to clarify the incentives associated with ITQs if we consider a hypothetical fishery in which all quotas are held by non-fishers and leased to fishers. Based on the preceding economic arguments, one would expect the quota holders to act collectively to manage the fishery to maximize the present value of the long-term profit stream. They should be prepared to invest in research, management, and enforcement to the extent that the pay-off in terms of increased present value exceeds the cost. (It is of course questionable whether a strict cost–benefit analysis would support any level of investment in research, management, and enforcement in small marginal fisheries, if costs are high and potential profits are small.)

Fishers who only lease quota have no long-term stake in the fishery (apart from practical constraints inhibiting relocation to other fisheries). They are required to bid competitively to lease annual quota and so face strong incentives to reduce costs and increase market value. However, they can expect the resulting gains in profitability to be translated into increasing quota value and lease prices. Individually, they stand to gain through quota-busting or discarding, unless the expected probabilities of detection and penalties are sufficiently high. However, in this respect they are in the same position as quota-holding fishers, given that the possession of a small fraction of total quota has negligible effect on the net gain from cheating.

If the state retained ownership of the total quota and leased individual annual quotas on a competitive basis to fishers, it would essentially be in an equivalent position to a group of private, nonfishing quota holders. Given that fisheries and the underpinning marine ecosystems are generally thought of as public resources and that management of these resources is generally required to take account of other public goods, including social and conservation objectives, the justification for transferring nonfishing quota from public to private ownership is debatable (Hilborn et al. 2005). Some might argue that private ownership leads inherently to greater economic efficiency, but it could be difficult to distinguish any inherent efficiency gains from the effects of ignoring social and conservation objectives. Nonfishing quota holders are no better placed than government agencies to gain access to information held by fishers (Arnason 2008).

In many ITQ implementations, there are rules that prevent or constrain quota ownership by non-fishers. It is certainly to the advantage of fishers to own quota, as they can expect to retain the economic benefits of increases in profitability due to good management and efficiency. However, it is not obvious that quota ownership by fishers necessarily translates into better management, either in economic terms (increased present value) or against other objectives. It might lead to increased collective sharing of information and more efficient assessment and management, as Arnason (2008) suggests. It could lead to lower enforcement costs, but if so, this must be ascribed to social factors or responses to individual profitability, given that the effects of quota possession on the benefits accruing from cheating are generally small. It would certainly be interesting to compare and contrast the level of compliance in ITQ-based fisheries between fishers owning vs. leasing quota.

There are alternative methods of allocating quotas, such as territorial use rights fisheries (TURFs) and cooperative and community quotas (Copes and Charles 2004; Branch et al. 2006*b*; Canchino et al. 2007). These methods assign non-transferable quotas to groups, who are then responsible for managing access to the quotas by individuals within the group. Groups may be chosen based on spatial subdivision or other factors and generally represent local and (or) traditional communities. Of course, these groups are then faced with the same issues faced by fisheries managers generally, albeit at a smaller scale (Canchino et al. 2007). How is entry to the group and access to the local resource controlled? Is access competitive, leading to a race to fish? Are returns

pooled, and if so, how are shirkers discouraged? Are individual quotas allocated within the group, and if so, are these transferable?

One might think of these alternative quota allocations as a way of downscaling the problems of fishery management to local levels. It is argued that the increased transparency and social cohesion in small communities leads to greater information sharing, discourages cheating, and reduces enforcement costs (Copes and Charles 2004; Gezelius 2004; Canchino et al. 2007). It may also create opportunities for co-management of multiple species for multiple purposes (e.g., commercial and recreational fishing and ecotourism, Canchino et al. 2007). Community-based management may not maximize economic efficiency, but proponents would attribute that to the higher priority assigned by communities to social objectives (Copes and Charles 2004).

It is also argued that allocations of nontransferable quota to communities are more equitable, since profits are retained by traditional users, rather than transferred through sale of quota. Some of the debate over ITQs is really about who should benefit from the profit stream resulting from an efficient, well-managed fishery. Those who see marine ecosystems and fish stocks as a public resource would argue the state should receive the excess profit stream as resource rent, which it could do by retaining and leasing quota. Traditional users of a resource (no matter how short the "tradition") tend to feel a sense of ownership or entitlement and expect to be given ownership of quotas. The state could choose to cash in the value of its resource ownership by a one-off sale or auction of quota. In practice, in most ITQ schemes, quotas have been given to fishers based on recent catch histories. That may represent a de facto recognition of the fisher's entitlement through prior use. Government agencies, faced with high recurrent management costs including periodic expensive buy-back schemes and unprofitable fisheries, may also see the handout of ITQ as a net gain for the state rather than a net loss.

As noted above, state agencies generally retain responsibility under ITQ management for monitoring, research, the setting of TACs, and compliance enforcement. In some cases, the associated costs are partly or wholly recovered from industry, while in others, these costs are met by the state (Newell et al. 2005; Sanchirico et al. 2006; Branch and Hilborn 2008). It is hard to see the justification for the state meeting these costs when the profit stream is directed to private quota holders.

Conclusions

The assignment of a long-term entitlement to future catches, in the form of an ITQ, does not remove the economic incentive for individual fishers to take additional unreported catch, even though the collective long-term outcome of such additional catches is likely to be a reduction in catches and profits. This prediction is robust, because it depends primarily on the fact that individual fishers can realise 100% of the immediate benefit of any additional catch, but spread the long-term cost among all quota holders. This is the "tragedy of the (local) commons", and it is only marginally affected by possession of small fractions of total quota.

This has been explicitly recognized in the ITQ literature, and strong and effective compliance monitoring and enforcement are widely accepted as essential to the success of ITQ-based management. Other social pressures and values are known to influence individual decisions about compliance, and these might be expected to have substantial effects on enforcement costs and management outcomes. The effect of possession of quota on compliance might be evaluated by comparing compliance levels in fisheries with varying levels of quota ownership by non-fishers.

It is clear that ITQs, with adequate enforcement, can change the incentives for individual fishermen so as to effectively eliminate the race to fish and increase profitability through reductions in overcapacity, improvements in fleet efficiency, and equally importantly, increases in market value of landed product. ITQs do pose particular issues of discarding and high-grading in multispecies fisheries, but there is evidence that these can be overcome with sufficient investment in monitoring and appropriate incentives and quota flexibility.

From an economic point of view, ITQs are intended to maximize the present value of the profit stream from fishing, and again there is evidence that they can be successful in doing that. The profit stream accrues to quota holders. Depending on the rules around ITQ eligibility, quota may or may not be restricted to fishers. There would appear to be a compelling argument that quota holders, who enjoy the benefits of the profit stream, should meet the costs of the monitoring, research, and enforcement required to sustain it.

There are other approaches that partition quota among groups (TURFS, cooperatives, communities), often on a spatial basis. These groups arguably face the standard fisheries management problems of allocation among individuals within the group, albeit at a smaller scale. Proponents argue that these methods enjoy the benefits of increased transparency and social cohesion at community scales and are better placed to balance social and economic objectives.

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ERRATUM / ERRATUM

Erratum: Diversity and complexity of angler behaviour drive socially optimal input and output regulations in a bioeconomic recreational-fisheries model

Fiona D. Johnston, Robert Arlinghaus, and Ulf Dieckmann

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In Table 1 in the printed and online versions of the article, there are typesetting errors in eqs. 2*a*, 2*c*, and 7*e*. The correct versions are as follows:

[2*a*] $p_{ij} = \exp(\widehat{U}_{ij})/[\exp(U_o) + \exp(\widehat{U}_{ij})]$
[2*c*] $D_j = p_{ij} D_{\max}$
[7*e*] $C_{ijp} = \min(C_p, c_{\max} \rho_p / \Psi)$.

Figure 1 in the printed version of the article contains production errors. The correct version appears on the following page.

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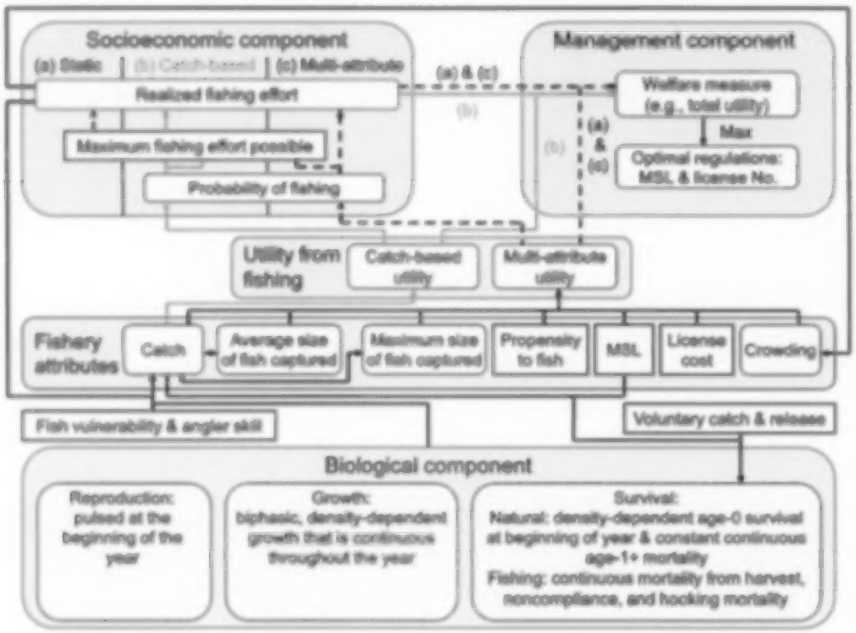
Fig. 1. Simplified flow diagram illustrating interactions among the three model components of our bioeconomic modelling approach: the biological component, the socioeconomic component, and the management component. The model included three angler-behaviour scenarios: (*a*) static angler behaviour, where anglers fished at the maximal rate, (*b*) catch-based dynamic angler behaviour, where anglers responded to the fishery based on catch rates, and (*c*) multi-attribute dynamic angler behaviour, where anglers responded to the fishery based on a multi-attribute utility function. Black solid arrows depict influences that apply across all scenarios, while gray arrows apply to the catch-based scenario only and black broken arrows apply to either the static or multi-attribute scenario, as is also indicated by labels beside the arrows. Factors in round-cornered boxes dynamically changed throughout model runs, while parameters for factors in square-cornered boxes were held constant.



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